Social Life-Styles in Caterpillars: Behavioral Mechanisms and Ecological Consequences

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Social Life-Styles in Caterpillars: Behavioral Mechanisms and Ecological Consequences

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Preface

Some remarks in advance…

This doctoral thesis follows the widespread international standard of PhD theses and is structured in independent chapters which together build a closed unity, yet can be read separately without having read preceding chapters (cumulative design). Nevertheless, accompanying chapters shall help the reader to gain further concomitant information and give the whole thesis coherence.

In Chapter one I give a general introduction on social caterpillars and a first overview of the present state of research as well as an outline of the thesis including the formulation of the questions and hypotheses addressed. Chapters two and three mainly include literature basics and show only few original data. They give an overview of the species investigated and some additional information on physical details of thermoregulation which might be helpful for those readers who have not yet dealt with this subject.

Chapters four to thirteen all show original data and follow the general appearance of scientific papers since the majority of these chapters has been published yet (Chapters 4,5,6,7) or are presently under review (Chapters 8,10,11). I decided to accompany each chapter with its own references. Although this means partial redundancy of references, readers who are only interested in partial aspects of this thesis benefit from the integrity of each chapter.

In a last chapter, the synopsis, I merge the contents of all chapters to provide general discussion and conclusions about the significance of the data for caterpillar sociality.

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Chapter 1

General Introduction

Present state of research

Butterflies and moths (i.e. the order Lepidoptera) probably represent the best known taxon among the phytophagous insects. However, there is a deep gap in our knowledge about the ecology of the predominant number of species which is not only true for tropical species, but even for many of the most common species of our local fauna. This particularly holds for the larval stages of most Lepidoptera. Hence, the larval ecology is only well understood for a rather small number of species which are of economic interest as they are forest or agricultural pests.

The larval stages of the majority of the about 160,000 described species of butterflies and moths show solitary life styles. In contrast, only about 300 species are known so far which exhibit gregarious or 'social' life-styles for at least part of their larval development (for a general discussion of terms see: Costa & Fitzgerald 1996, Costa & Pierce 1997). Even though this list is certainly incomplete because of the nearly complete lack of knowledge of life-history traits of most tropical species it seems obvious that evolution favors solitary life-styles in most cases investigated because of the high costs related to social life.

The costs of larval societies are obvious: Caterpillars are slow moving insects and suffer great losses from predators and parasitoids which could be even more pronounced in groups of larvae that are more conspicuous to their enemies (Costa 1993, Knapp & Casey 1986, Stamp & Bowers 1988). Besides, groups of caterpillars have a higher risk of being infected by pathogens (Hochberg 1991) and may rapidly overexploit their larval resources because of competition for food within a colony which may result in the inability to finish development and sometimes the death of the whole offspring of a female (Tsubaki 1995).

Five factors acting singly or in combination are commonly regarded to be responsible for the fact that despite strong selective pressures against larval communities such systems may evolve and persist over time:

1. The inability of the imago to ingest food favors egg clustering

Comparative investigations revealed that a reduction of the proboscis in imagines frequently makes for clustering eggs in large clutches because of energetic constraints (Miller 1996, Tammaru & Haukioja 1996, Hebert 1983). Caterpillars hatching from an egg cluster immediately leave their siblings in most species. Nevertheless, egg clustering is obligatory for evolving social life-styles in caterpillars and gregarious larvae are significantly more frequent in species that do not feed as adults (Hunter 1991).

2. Aposematic coloration and collective defense against natural enemies is more effective in groups

Aposematically colored caterpillars that are generally supposed to be unpalatable have an advantage over solitary caterpillars because of enhanced signaling efficiency and more rapid avoidance learning by predators (Gagliardo & Guilford 1993, Gamberale & Tullberg 1998). Aggregations therefore can be found especially in aposematically colored caterpillars (for butterflies: Sillén-Tullberg 1988, 1993). In addition, some caterpillars show special group defense displays (e.g. synchronized regurgitation) and are thus able to deter invertebrate predators and parasitoids more effectively than solitary individuals (Peterson et al. 1987, Stamp 1984).

3. Possibility to build large silk webs

Some social caterpillars build silken structures that can be formed as loose webs or dense ‘tents’ (cf. Costa & Pierce 1997). Webs or tents are multifunctional structures with different tasks such as giving shelter from desiccation, or protection from invertebrate predators and parasitoids (Stamp 1981). Furthermore the tent may transfer vibrations which could enhance synchronized group behavior (Costa 1997) and serve as effective material for thermoregulation (Breuer & Devkota 1990, Joos et al. 1988) (cf. point 5).

4. Social feeding facilitation

Social behavior can be advantageous for foraging in many ways which include communal overcoming of plant defense structures (Clark & Faeth 1997, Denno & Benrey 1997, Matsumoto 1989), optimal exploitation of resources (Tsubaki & Shiotsu 1982), or (in the case of patchily distributed food resources) the development of highly sophisticated, pheromone based communication systems which enable caterpillars to find high quality food resources quickly (Fitzgerald & Peterson 1988, Peterson 1987 and references therein). Indeed, the latter may also be a consequence of overexploitation of food resources in large groups of caterpillars. This means that highly evolved communication systems may rather have evolved as a consequence of social behavior and did not initiate it.

5. Thermoregulation in groups

Solitary caterpillars are able to elevate their body temperature by exposing their preferably dark colored bodies to the radiation of the sun, which enhances digestion rate and consequently the developmental rate (Casey et al. 1988). Temperature gains are usually only in the range of a few Kelvin. In aggregations, caterpillars multiply their effective body mass which results in much larger temperature gains than in solitary larvae (Casey 1993, Knapp & Casey 1986, Stevenson 1985a,b). Furthermore, if caterpillars build tents, these structures may enhance temperature gains even further (Breuer et al. 1989, Casey et al. 1988, Joos et al. 1988). Nevertheless, the physical features of such tents have almost never been tested experimentally and thus the real mechanism of the heating function of the tent remains unclear. It is noticeable that sociality in caterpillars combined with tent building predominantly occurs in species that develop in early spring or late autumn where thermal conditions are very restrictive for ectothermic insects.

Larval societies are thence communities of mutual benefit for all group members driven by morphological constraints of the adults or by ecological constraints directly affecting development and survival of the caterpillars. Caterpillar societies will only evolve or persist if every individual within the group may benefit from the presence of the colony mates and therefore from its

staying in the colony (Hamilton 1971). In contrast to the well known social systems of 'eusocial' insects (e.g. ants, bees, or termites) aspects of kin selection (Hamilton 1964) are of no importance for larval societies. Kin discrimination seems to be non-existent in social caterpillars (Costa & Ross 1993) and intraspecific as well as interspecific mixed colonies regularly occur in the field (Fitzgerald 1995, Ebert 1994) or can easily be maintained in the laboratory. Studying social communities which are not determined by reproductive division of labor may help to analyze extrinsic ecological factors like advantages of communal search for food, defense against natural enemies or thermoregulation and to assess their importance for the evolution of social systems.

The factors promoting sociality in caterpillars defined under point 1 are usually investigated by comparative phylogenetic studies. In contrast, factors mentioned in points 2 to 5 permit the formulation of clear hypotheses, testing them experimentally and thus make advantages or costs of social life-styles measurable.

Although there is a considerable amount of studies on different lepidopteran species with social caterpillars (for a general review see Fitzgerald 1995, Costa 1997), most studies deal with one single aspect of the caterpillars' pattern of life (e.g. enhanced survival in gregarious larvae). Only chemical aspects of trail-based communication were studied more intensively in several species, but only one species has been investigated very well, the highly social caterpillars of the Nearctic eastern tent caterpillar *Malacosoma americanum* (Lepidoptera, Lasiocampidae) (reviewed in Fitzgerald 1995). Nevertheless, even after over 30 years of research numerous questions remain to be answered in this particular species of considerable economic importance.

Levels of complexity in caterpillar sociality

Unlike eusocial insects caterpillars do neither exhibit cooperative brood care, nor reproductive division of labor, and there is no contact between generations. However, as in any other social systems the complexity of sociality is rather

different between species. Although a lot of factors may be useful to categorize social caterpillars the classification by foraging behavior has become widely accepted and will therefore be used in this thesis. Of course, social caterpillars use a wide variety of foraging strategies that build a continuum. Nevertheless it seems useful to broadly classify them as either patch-restricted, nomadic or central-place foragers (Fitzgerald & Peterson 1988; Fig. 1).

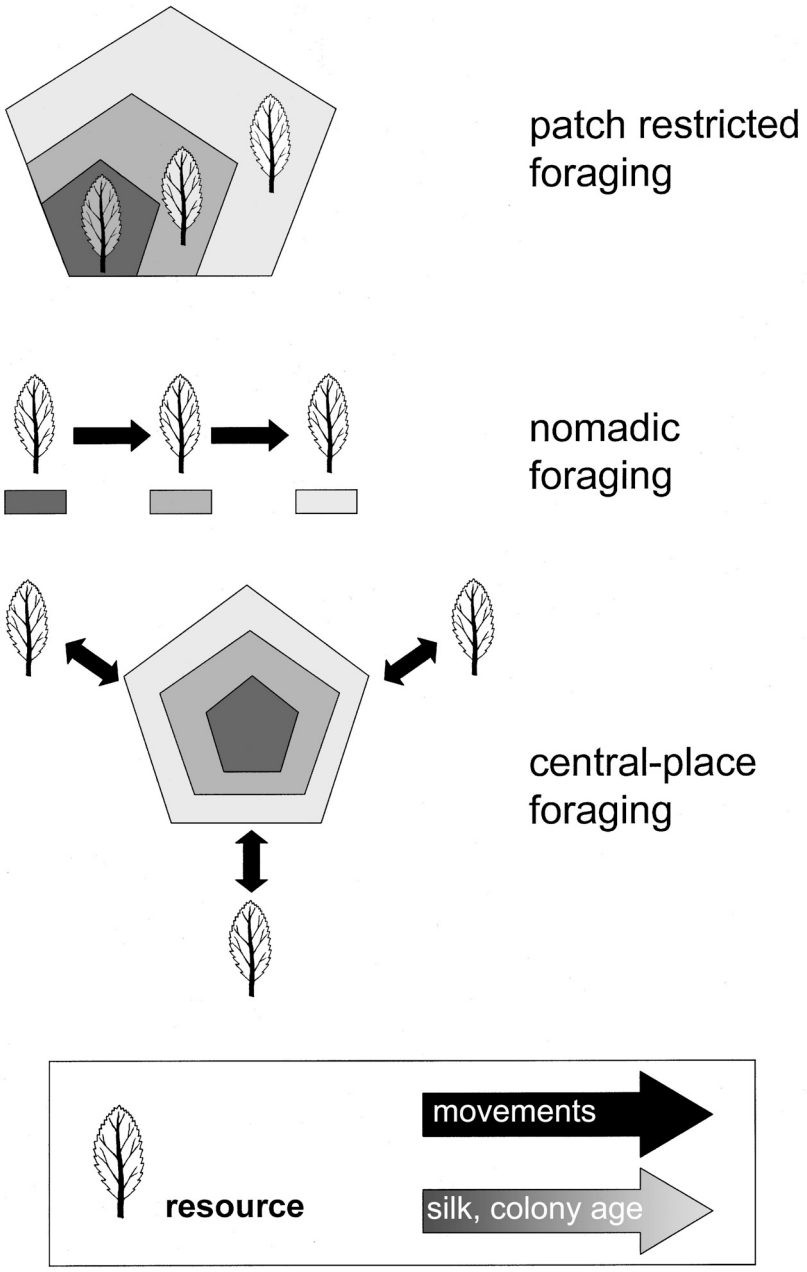

Fig. 1 – Manifestation of sociality in caterpillars. Terminology after Fitzgerald & Peterson (1988).

Patch restricted foragers represent the simplest form of sociality. These species usually stay at the same location throughout the larval stage, typically within constructed shelters and feed on leaves incorporated within these webs. Webs are continually expanded so that sometimes large composite webs emerge that cover entire trees or hedgerows (e.g. Hoebke 1987). Communicative abilities are low and mainly serve group cohesion.

Nomadic foragers move in groups from one food patch to the next. Depleted sites are regularly abandoned. Nomads do not construct three-dimensional silken shelters but often build silken pads for resting or molting. Communication is used for cohesion, group defense and the spatial orientation between feeding and resting sites.

The most complex system of sociality in caterpillars is represented by central-place foragers. These species usually construct shelters but unlike patch restricted foragers they feed outside these constructions in the surrounding vegetation. As feeding sites become more and more scattered during the course of the caterpillars' development, it is indispensable to evolve a sophisticated communication system which includes group cohesion, group defense, spatial orientation, and the exchange of information about feeding-sites.

Research gaps and outline of this thesis

Much research remains to be done by studying further species of social Lepidoptera to gain deeper insight in the general validity of some conclusions drawn from the study of only few 'model' species. Furthermore, some interesting topics that remain virtually untouched need to be studied in more detail.

In order to benefit from some advantages provided by sociality, e.g. thermoregulation, communal tent building and group foraging, it seems crucial for caterpillars to synchronize their activity (Casey et al. 1988). Synchronized activity periods and foraging bouts have been studied in *Malacosoma americanum* (Fitzgerald 1980, Fitzgerald et al. 1988) and in the caterpillars of

the European small eggar moth, *Eriogaster lanestris* (Lasiocampidae) (Ruf et al. 2001) but these studies were thus far limited to the description of foraging patterns. Thus, it is crucial to understand how caterpillars manage to synchronize or coordinate their activities. Synchronicity necessarily implies costs, because needs of individuals (e.g. feeding) must be suppressed temporarily. Furthermore, analyses of more species are necessary to test if behavioral synchronicity is really essential for social caterpillars.

Several studies have dealt with the benefits of using a tent for behavioral thermoregulation (Breuer & Devkota 1990, Joos et al. 1988) and thermal budgets of single and grouped caterpillars have been analyzed in detail. It was suggested that the tent may function like a greenhouse (Joos et al. 1988) but the physical features of the tent have never been investigated and the spectral features like transmittance of the silk layers are almost totally unknown.

Thermoregulation in ectotherms is generally supposed to be bound to the absorption of solar energy, but hints in the literature allow for the assumption that there also a metabolic component of social thermoregulation in caterpillars could exist (Mosebach-Pukowski 1937). However, this hypothesis has never been tested experimentally.

Against this background the present work has three main purposes and aims:

1. Description and measurement of **advantages** of social life-styles and enhancement of existing knowledge on caterpillars of other species. This includes measurements of physiological and ecological consequences of social behavior like thermal budgets of social caterpillars under different climatic conditions as well as investigations of life-history traits of different species and group size effects.

2. Analysis of **ethological preconditions** which make it possible to benefit from these advantages. Here I attach special importance to the synchronicity of foraging bouts and its influencing factors as well as communicative abilities of the caterpillars.

3. Analysis of **mechanisms** to understand *how* behavioral patterns work, or which physical background is responsible for the measurements made. For this

purpose I investigated several aspects of trail following and trail marking to understand how decision making in individuals works and how the trail marker is applied. Furthermore I analyzed physical features of the silk of the tent (see above) and surveyed the temporal change in vibrations of the tent to better understand mechanisms underlying synchronized foraging bouts.

The main emphasis of this thesis I put on the investigations of the social caterpillar of the small eggar moth *Eriogaster lanestris*. The small eggar is one of the few species with a highly evolved social system. Its larvae stay together until the end of the last instar and leave their tent only a short time before pupation (Pro Natura 2000). *E. lanestris* is well comparable with *M. americanum* with regard to life history (univoltine, early spring species), tent construction (multi-layered, dense silk structure), host plant requirements (polyphagous on several shrubs and trees) and group size (female lays all its eggs in one cluster). One chapter of this thesis also focuses on a comparison of *E. lanestris* with its congener *Eriogaster catax* and the confamilial species *Malacosoma neustria*.

In addition to these highly social, central-place foraging species I furthermore investigated caterpillars of the map butterfly *Araschnia levana*. Social cohesion is weak in this species and caterpillars only stay together until the end of their third instar. They build no tent, do not thermoregulate communally, and aggregate on the underside of the leaves of their only host plant, the nettle, *Urtica dioica*. Groups are smaller as females only allocate parts of their egg load into each egg cluster. Therefore, I expect advantages of sociality to be less pronounced in this species.

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Chapter 2

General life-history traits of the species investigated

In this thesis four different Lepidopteran species were used for the experiments. The first three (*Eriogaster lanestris*, *Eriogaster catax*, and *Malacosoma neustria*) belong to the moth family Lasiocampidae, whereas the fourth species (*Araschnia levana*) belongs to the butterfly family Nymphalidae.

The most relevant feature concerning larval sociality (cf. previous chapter) is that all Lasiocampidae lack a functional proboscis and are thus not able to feed as adults. They totally rely on the energy reserves built up during their larval phase and adult moths are therefore very short-lived. Females often live less than one day since they die immediately after egg deposition which often occurs in the first night after eclosion. For a general overview of the family see Common 1990, deFreina & Witt 1987, Lemaire & Minet 1998, Scoble 1992.

In contrast, adult butterflies of *Araschnia levana* have a functional proboscis and can often be seen nectaring on flowering plants. Under laboratory conditions (L:D 18:6h, 21°C from 10:00-17:00h, otherwise 12°C) mated females may thus live up to 35 days (mean longevity±SD=24.8±6.8d, N=17) (data from first laboratory bred generation. C. Ruf, unpubl. data).

Below, I describe general life-history traits of the four species under consideration. All species are shown on color plates at the end of this chapter.

Eriogaster lanestris (Linnaeus, 1758)

The Western Palaearctic distribution of the Small Eggar, *Eriogaster lanestris*, ranges from the North of the Iberian peninsula over Southern, Western and Northern Europe, through Asia Minor to the South of Russia (de Freina 1999, Ebert 1994). In the East the species ranges to Kazakhstan, Southern Siberia, Jakutsk and up to the Amur region (Dubatalov & Zolotuhin 1992).

Eriogaster lanestris is strictly univoltine. Moths hatch in early spring (usually March to April in Central Europe) and caterpillars can be found predominantly in May and June. Mated females lay one single cluster of eggs which is wound several times in a close spiral around small twigs of the host plant (see below). As the eggs are laid the hairs from the female's anal tuft are shed, but in such a way that the hairs stand out vertically from the egg mass, which, when completed, suggests a small piece of fur encasing the twig. This type of egg cluster is typical for all species of the genus *Eriogaster* (cf. Bolz 1998, Ortner 1994, ProNatura 2000, Tham et al. 1986, Trawöger 1977, Talhouk 1940, 1975) and is not known from any other Holarctic members of the Lasiocampidae.

Caterpillars hatching from the egg cluster start building their home web or tent at once, normally directly around the cluster which becomes the middle of the tent. This tent is continuously enlarged over the course of the caterpillars' development. *E. lanestris* is a typical 'central-place forager' (term according to Fitzgerald & Peterson 1988). The tent is used for resting and molting and does not include any resources. Thus, caterpillars have to leave the tent whenever they want to take up food. Caterpillars remain social until the end of their last (fifth) instar when they finally leave the tent in search of a place for pupation. Prepupal *Eriogaster* caterpillars dramatically shrink to about one third of their maximum size and build a hard barrel-like cocoon which is interspersed with the reddish urticating hairs that can cause severe contact dermatitis (so-called erucism) (Hellier & Warin 1967). The pupa overwinters at least once. Emergence of the moth may be delayed for several years (Carlberg 1980, Van Nuvel 1976, Balfour-Browne 1933).

In Europe the main habitat of the Small Eggar consists of either xeric habitats with the host plants blackthorn (*Prunus spinosa*) or hawthorn (*Crataegus* sp.), or former peat bogs with stands of birch (*Betula* sp.). Besides, this polyphagous species has been found on lime (*Tilia* sp.), willow (*Salix* sp.) or other deciduous trees and shrubs (Linné 1767, Ebert 1994, ProNatura 2000). Larval tents exclusively occur in sun-exposed conditions and never in the understorey of closed forests.

This once common moth is a declining and very local species nowadays, occurring in widely scattered populations throughout Europe. At the turn from the 19th to the 20th century this species has declined, now being absent from many habitats where it once was common. The large-scale destruction and indiscriminate annual trimming of hedgerows, on which it relies for its host plants and larval habitat, combined with the pollution caused by motor-vehicles (Stary et al. 1989) and the drift of agricultural insecticides, have all contributed to the serious decline of this species. Nevertheless, the Small Eggar has only premonition status in Germany's Red Data Book for endangered species (Bundesamt für Naturschutz 1998), but is listed with different status of endangerment in several 'Bundesländer' (Germany's federal states) in their regional Red Data Books, for example Schleswig-Holstein, Niedersachsen and Mecklenburg-Vorpommern (Kolligs 1998 and references therein).

Eriogaster catax (Linnaeus, 1758)

In contrast to *Eriogaster lanestris*, this species shows a far more restricted distribution and ranges from Northern Spain over Central Europe (including the Balkan but excluding the northern countries) to the Ural and Asia Minor (ProNatura 2000). This species which lives in open deciduous forests of broad-leaved trees saw its habitat reduced by afforestations of coniferous trees and the giving up of traditional forms of forest cultivation like coppice or coppice with standards. In these old forms of forest cultivation, cyclic logging of small areas leads to a mosaic of different succession phases with blackthorn bushes in the understorey and large oaks in between that are not lumbered. *E. catax* already disappeared from Luxembourg, is not observed any more in Italy and since 1950 is declining in Hungary and is restricted to few isolated populations in Germany and Switzerland. As a consequence of its severe endangerment throughout Europe *E. catax* is listed among the endangered species of special concern for the European Community as a species that requires the designation of special areas of conservation and which is in need of strict protection (Appendix II, IV: Council directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora).

Despite its conservation status reliable information on any aspects of the biology of *E. catax* is extremely scarce. In contrast to *E. lanestris* moths hatch in autumn (September, October) and eggs overwinter. *E. catax* moths are more sexually dimorphic with the males being colored yellowish brown or nearly orange whereas females are of a darker brown. Eggs are covered with the hairs from female's anal tuft as described for *E. lanestris*.

The social phase of *E. catax* is shorter than in *E. lanestris* and caterpillars disperse in an earlier instar, but statements in the literature about the time when caterpillars leave their aggregations differ between 'after the second molt' (i.e. the beginning of the third instar) (ProNatura 2000) and 'in the last (i.e. fifth) instar' (Bolz 1998).

Food plants are various deciduous trees and shrubs including blackthorn (*Prunus spinosa*), willow (*Salix* sp.), poplar (*Populus* sp.), and oak (*Quercus* sp.).

Malacosoma neustria (Linnaeus, 1758)

In contrast to the genus *Eriogaster* which is restricted to the Palaearctic, the genus *Malacosoma* has a broader, Holarctic distribution with about 20 species in the Palaearctic and another six species in the Nearctic (Fitzgerald 1995 and references therein). Despite the fact that larvae of all species of *Malacosoma* are social, yet do not always build a silken tent, they are commonly referred to as 'tent caterpillars'.

The distribution of *Malacosoma neustria*, the Common Lackey, or European Lackey Moth, is wide. It has been recorded for the European countries and all larger Mediterranean islands (Karsholt & Razowski 1996) and North Africa (de Freina & Witt 1987). Eastwards the species ranges throughout the entire Palaearctic region but Asiatic populations are commonly referred to as another subspecies (i.e. *M. neustria testacea*).

Moths hatch in summer (July in Central Europe: Ebert 1994) and females lay their eggs in a cluster in one closed, 'bracelet-like' ring around the twigs of their host plants which has given rise to the German name 'Ringelspinner' (i.e.

Ringel = annular structure; Spinner = bombycoid moth). Since females do not possess an anal hair tuft, eggs remain visible. As the eggs are deposited they are held in place by a liquid substance from the accessory glands which has been named 'spumaline' by Hodson and Weinman (1945) and which hardens very soon.

Caterpillars hatch in early spring and build a silken tent. They stay together until they molt into the final instar (Tutt 1900, ProNatura 2000). Cocoons are elliptic and soft and contain a yellowish powder (see Tutt 1900 for details). Pupae are much taller than in *Eriogaster* (cf. Patočka 1984) and are very agile within the cocoon.

Caterpillars are highly polyphagous and can be found on several trees and shrubs including *Quercus*, *Betula*, *Populus*, *Tilia*, *Prunus*, *Crataegus*, *Rubus*, and *Rosa*. *M. neustria* is a species of economic impact for the timber industry since it is known to defoliate several hundreds of hectares per year, especially during outbreaks (e.g. Csóka 1998). *M. neustria* has once also been a pest of fruit trees in Central Europe (Tutt 1900) but no larger outbreaks have been reported during the 20th century (Ebert 1994). Presently the species is still widespread in Central Europe but its abundance tends to decline.

Araschnia levana (Linnaeus, 1758)

The European Map Butterfly, *Araschnia levana* (Nymphalidae), is well known for its striking seasonal polyphenism (Plate 4). The wing color of adults of the spring generation (f. levana) is reddish with a black pattern, whereas wings of butterflies of the summer generation (f. prorsa) show white stripes on a black ground color with only slight orange stripes that can also be completely missing (Ebert & Rennwald 1991). Occasionally, a third generation may occur in late autumn (f. intermedia) which is intermediate between spring and summer generation with changing portions of red or black scales (e.g. Carnier 1995). In the northern regions of its range and at higher altitudes the summer brood is often missing (Korshunov & Gorbunov 1995).

The polyphenism is primarily controlled by day length and can be modified by temperature. A short photoperiod induces diapause pupae which release the red spring form after hibernation, whereas a long photoperiod induces subitaneous development of pupae releasing the black summer form (Koch 1996, Müller 1992, Reinhardt 1969). The color of pupae varies from a pale to a dark brown with changing portions of shiny golden areas (cf. Neville 1977).

The physiological background of the determination of diapause or subitaneous development is well understood. Non-diapause larvae are characterized by an earlier increase of hemolymph ecdysteroid and an earlier pupation compared to diapause pupae. In addition the juvenile hormone titer of non-diapause larvae increases before pupation whereas that of diapause pupae remains low (Koch 1996, Koch & Bückmann 1987). The reddish color of the spring form is formed by an ommatin (dihydroxanthommatin), derived from tryptophan over the precursor 3-hydroxykynurenine (Koch 1991, 1993). In contrast, the black or brown color is caused by synthesis of melanin (derived from tyrosine) in the wing scales (Nijhout & Koch 1991).

Besides this striking polyphenism butterflies of the genus *Araschnia* are unique for their egg laying behavior: eggs are clustered by building vertical columns (‘turrets’) with eggs arranged in a pile one over the other on the underside of the leaves of its host plant, the stinging nettle (*Urtica dioica*) (Korshunov & Gorbunov 1995, Ebert & Rennwald, 1991, Fukuda et al. 1991, Plate 4).

Caterpillars hatching from the egg clusters remain gregariously until the end of the third instar when groups start subdividing into smaller groups, and finally the larvae become solitary. During their gregarious phase caterpillars do not build a tent but live nomadically on the underside of leaves of their host plant and move from one leaf to the next as soon as resources are exhausted. Caterpillars are colored uniformly black but the degree of melanization varies and larval color may range from a pale gray to a deep black (Windig 1999). The larvae are covered with black, reddish, or light-brown spines that are branched in later instars. The most striking spines are situated on the head capsule and give the caterpillar its devil-like appearance.

Caterpillars can easily be assigned to the five larval instars by measuring the width of the head capsule (Table 1).

Table 1. Width of head capsule of larvae of *Araschnia levana*. For first to fourth instar head capsules discarded during molting were used, last instar head capsules were measured directly at the living caterpillars which explains the slightly higher variation due to inaccuracies of the measurement. Sample size: N=10 for all categories.

Larval instar	L1	L2	L3	L4	L5
Width of head capsule, mean±SD [mm]	0.45±0.01	0.68±0.01	1.08±0.02	1.55±0.02	2.19±0.06

Araschnia levana ranges throughout the Palaearctic region from the Atlantic coast in the West to the Russian Far East and Japan (Fric & Konvička 2000 and references therein). The butterfly is relatively common in Central Europe. However, it is not as widely distributed as other nettle feeding nymphalids and is absent from the British Isles, Scandinavia and Finland. In Russia, the northern boundaries are commonly south of 60°N. The species is also absent from the Mediterranean regions, the Balkan and Turkey. Current records of Macrolepidoptera repeatedly indicate a northward expansion of the range of this species (e.g. Kaaber 1984, Koutroubas 1991, Palmquist 1996, 1998, Radigue 1994-95, Sala et al. 1996, Parmesan et al. 1999). The habitats preferred by *A. levana* are moist deciduous forests, especially edges and openings in light woods with rich nectar sources, river valleys and meadows adjoining rivers (Ebert & Rennwald 1991).

Barely nothing is known about the larval behavior of *A. levana* and implications of its social behavior are not understood at all.

Color plates

Legends:

Plate 1 (next page):

Life-stages of *Eriogaster lanestris* **A** female **B** female, anal tuft of hairs at the rear end of the body visible **C** male **D** egg cluster with newly hatching caterpillars **E** 'tent' with caterpillars, 2 days old **F** cocoon **G** opened cocoons with pupae **H** tent with basking 2nd instar caterpillars **I** trail following behavior on 5β-cholestane-3-one **J** foraging caterpillars **K** last instar larva **L** last instar larva, front part.

Plate 2 (page 20) :

Life-stages of *Eriogaster catax* **A** moth, male **B** moth, female **C** egg cluster **D** caterpillar, first instar **E** tent with 2nd instar caterpillars **F** caterpillar, 4th instar **G** caterpillar, 5th instar **H** caterpillar shortly before pupation **I** cocoons **J**,**K**,**L** tents with caterpillars of different age **M** fully grown larva.

Plate 3 (page 21):

Life-stages of *Malacosoma neustria* **A** male, pale form **B** male, dark form **C** female **D** prepupa **E** cocoon **F** opened cocoon, pupa **G** egg cluster **H** young caterpillars, foraging **I** old caterpillars, resting on branch **J** egg cluster (left), primary tent (further right) and L1 larvae on secondary tent (right) in the field **K** young larvae on tent **L** old larvae on tent **M** last instar larva, front part.

Plate 4 (page 22):

Life-stages of *Araschnia levana* **A,B** spring form **C,D** summer form **E,F** intermediate forms **G** prepupa **H** pupa **I** group of egg turrets on underside of a leaf of the stinging nettle, colored grayish shortly before hatching of the caterpillars **J** foraging caterpillars **K** caterpillars changing their feeding location **L** group of resting caterpillars **M** caterpillar, 5th instar.

Plate 1: *Eriogaster lanestris*

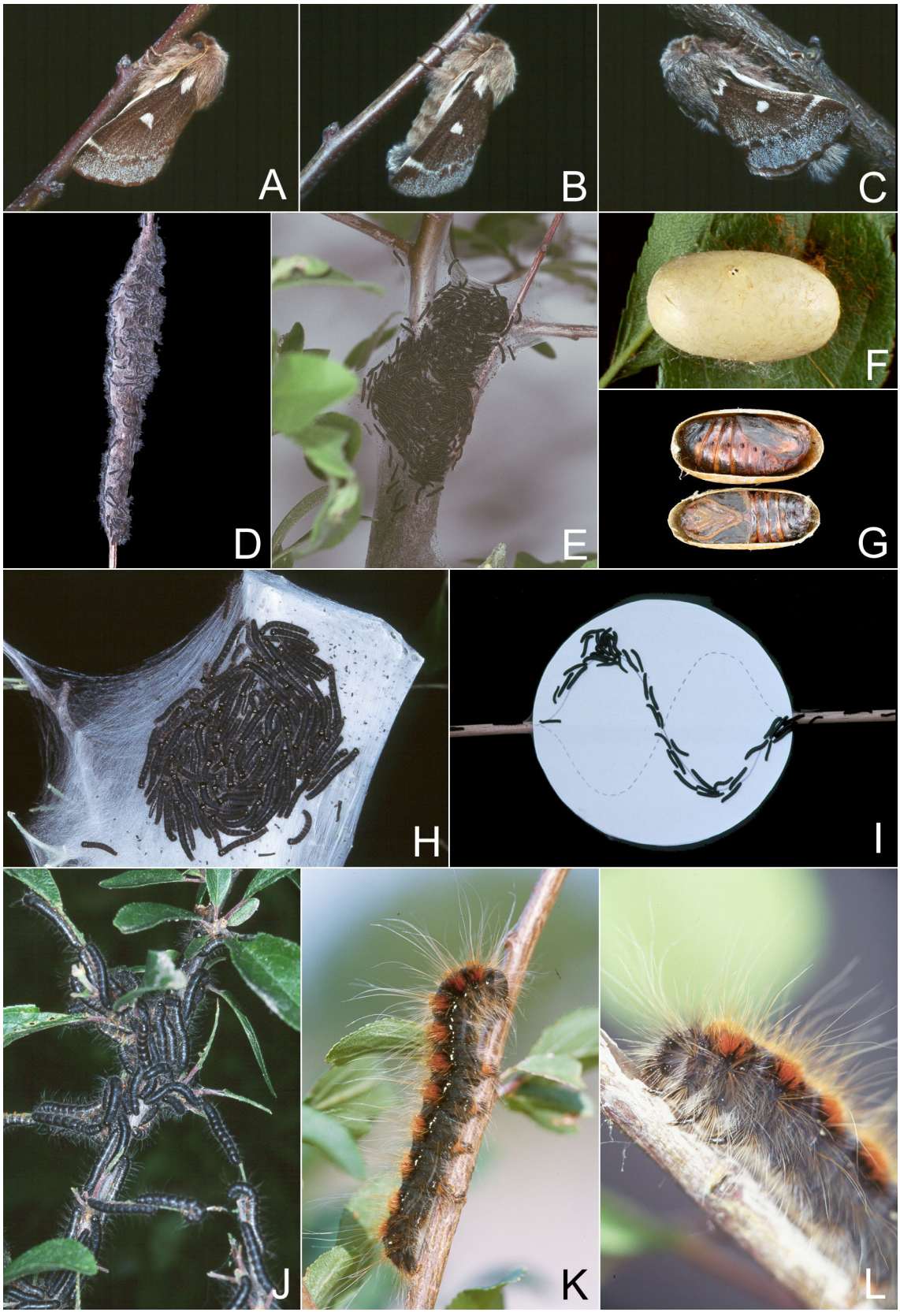


Plate 2: *Eriogaster catax*

Plate 3: *Malacosoma neustria*

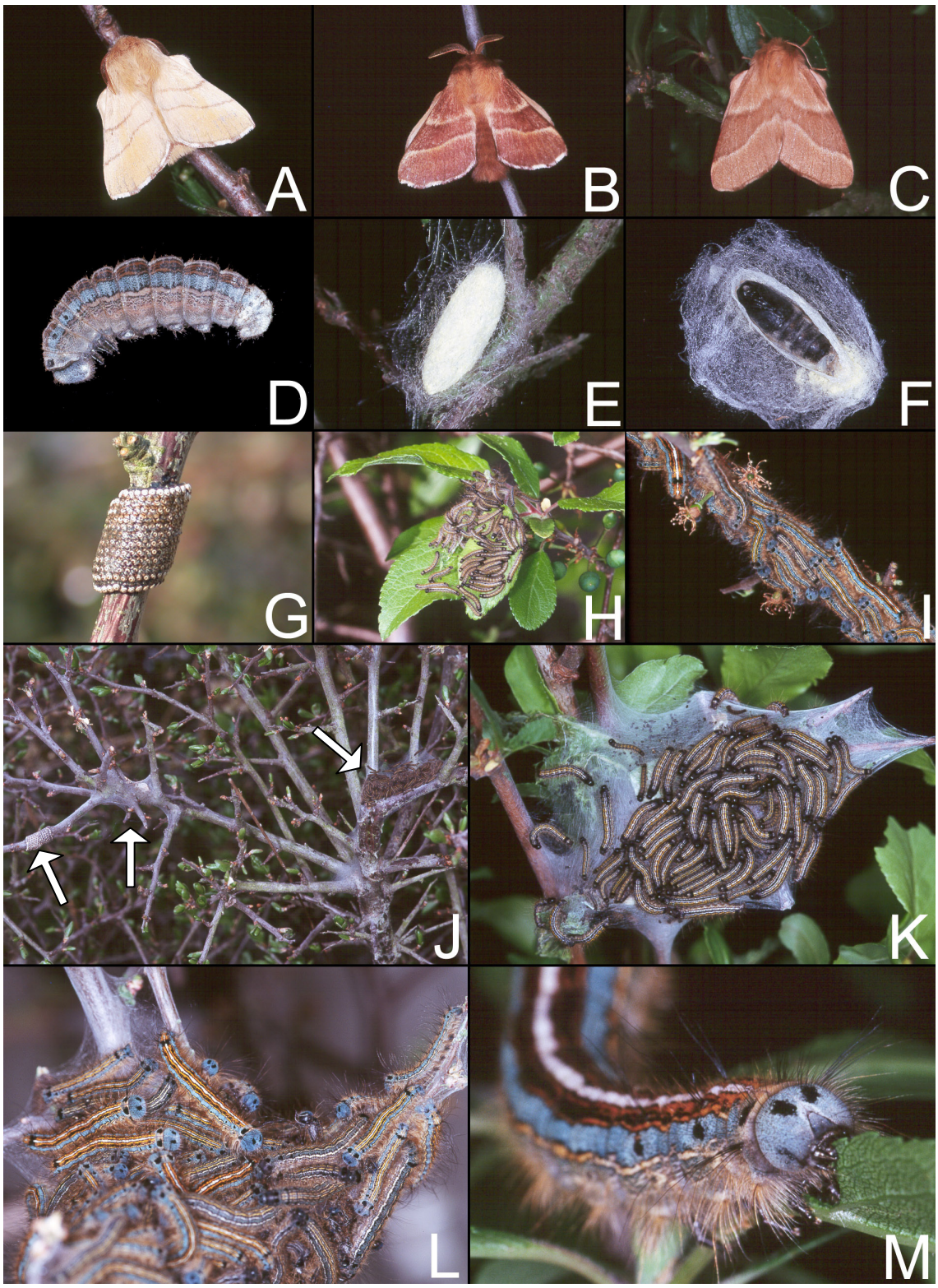
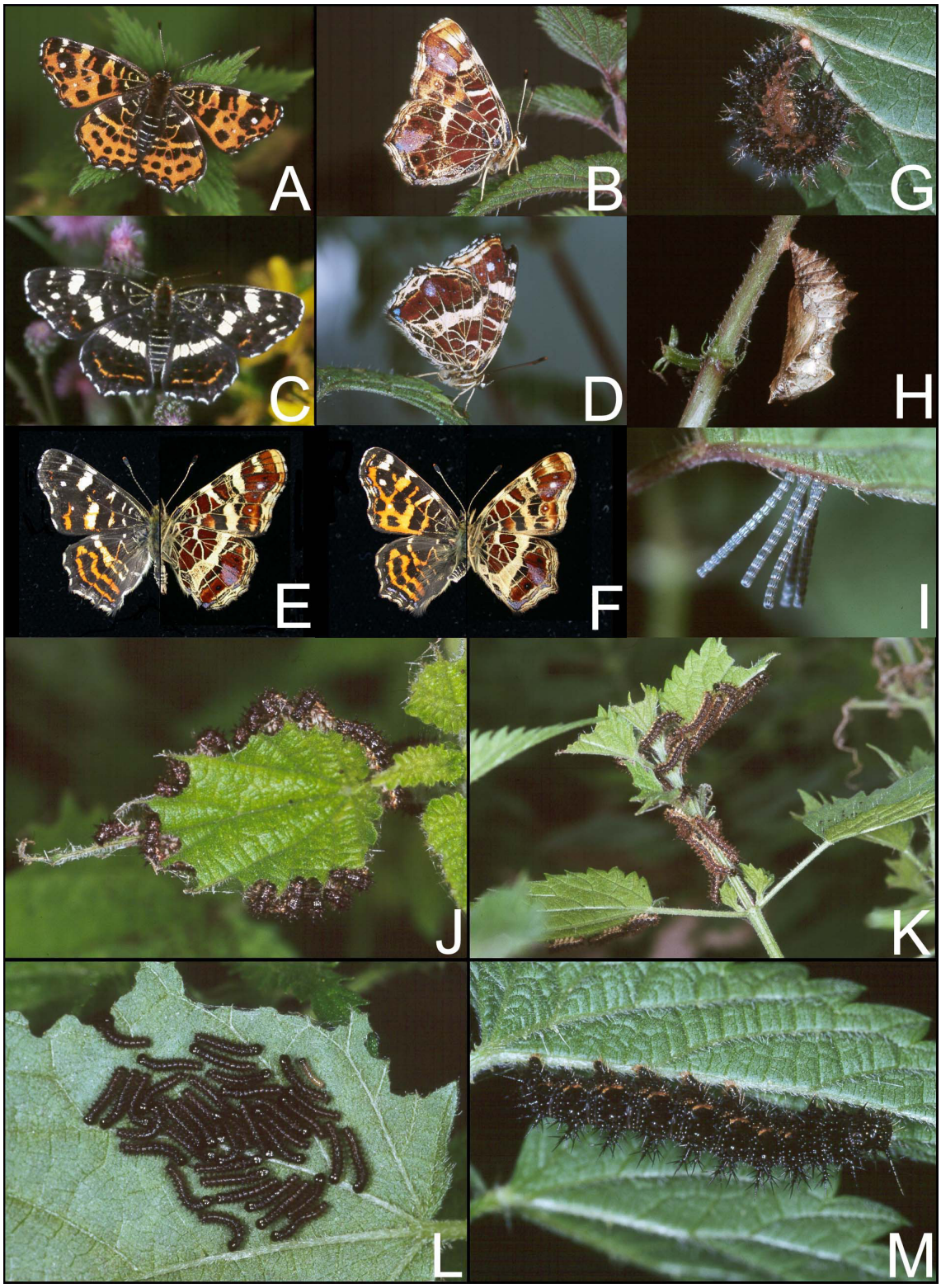


Plate 4*: Araschnia levana*



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Chapter 3

Physical basics of thermoregulation

In order to understand the processes that are important for thermoregulation in caterpillars it is necessary to introduce some terms and physical basics of thermodynamics and heat transfer. All definitions and numerical values in this chapter and thereafter follow Bayley et al. (1972), Hagen (1999), and Rolle (2000).

Heat transfer is the transport of thermal energy from one region to another. In order for heat transfer to occur, there must be a temperature difference between the two regions. Heat transfer occurs as an exchange of internal energy from one region to the other by atomic or molecular motion or by electromagnetic waves. The first law of thermodynamics requires that the energy given up by the hot object equals the energy gained by the cold object. The second law of thermodynamics requires that the natural direction of heat flow is from the hot object to the cold object. Another way to say this is that heat flows in the direction of decreasing temperature.

Heat transfer can occur in three distinct modes, namely conduction, convection, and radiation. Although in many practical situations – which is also true for the case of thermoregulating caterpillars – all modes of heat transfer occur together, it is usual to consider conduction, convection, and radiation separately.

Conduction

Conduction is the transfer of thermal energy in solids or fluids at rest. The actual mechanism of conduction involves kinetic energy exchange between molecules in contact or, in the case of metals, the movement of free electrons. The energy level of the elementary particles is a function of temperature, and thus as these particles move to regions of lower temperature they give up their excess energies.

Convection

Convection is the mode of heat transfer which occurs as the consequence of the motion of a fluid. The motion of the fluid arises from the difference in density between the heated fluid on the hot surface and the cooler, and therefore denser, surrounding fluid. The transfer of heat through the motion induced by the intrinsic volume or density changes associated with temperature differences in a fluid is known as *natural* or *free* convection. On the other hand, the rate at which heat is transferred as a result of externally induced fluid motion ('wind') is known as *forced convection* and is of great importance for thermoregulation under field conditions.

(Thermal) radiation

Radiation is the mode by which thermal energy is transferred by electromagnetic waves. Thermal radiation is just one manifestation of the wide spectrum of natural phenomena known as electromagnetic radiation. Electromagnetic radiation includes visible light, X-rays, γ-rays, ultraviolet, infrared and even radio waves (Fig. 1).

Unlike conduction and convection, radiation does not require a medium. A familiar example of radiation is the thermal energy that we receive from the sun across the vacuum of space. Actually, all objects with a temperature above absolute zero (0K) radiate thermal energy. As the temperature increases, the rate of energy emission also increases, and the peak of the emission distribution shifts to shorter wavelengths. Bodies or surfaces which absorb all radiation and interchange radiant energy at maximum rates are commonly known as black bodies. The black body is an ideal thermal radiator or absorber, and although there is no real surface that is a true black body, some surfaces can be well approximated as black bodies. The term black body does not mean that all black surfaces (as perceived by a human observer) are black bodies in a physical sense, nor does it mean that only black surfaces are black bodies. Some surfaces that are colored black do show some characteristics of black bodies; however, surface color does not determine whether a surface can be

approximated as black body. To get an idea of the range of wavelengths emitted, a black body at room temperature (20°C = 293K) emits radiation >2μm with a maximum of its emission spectrum at 10μm.

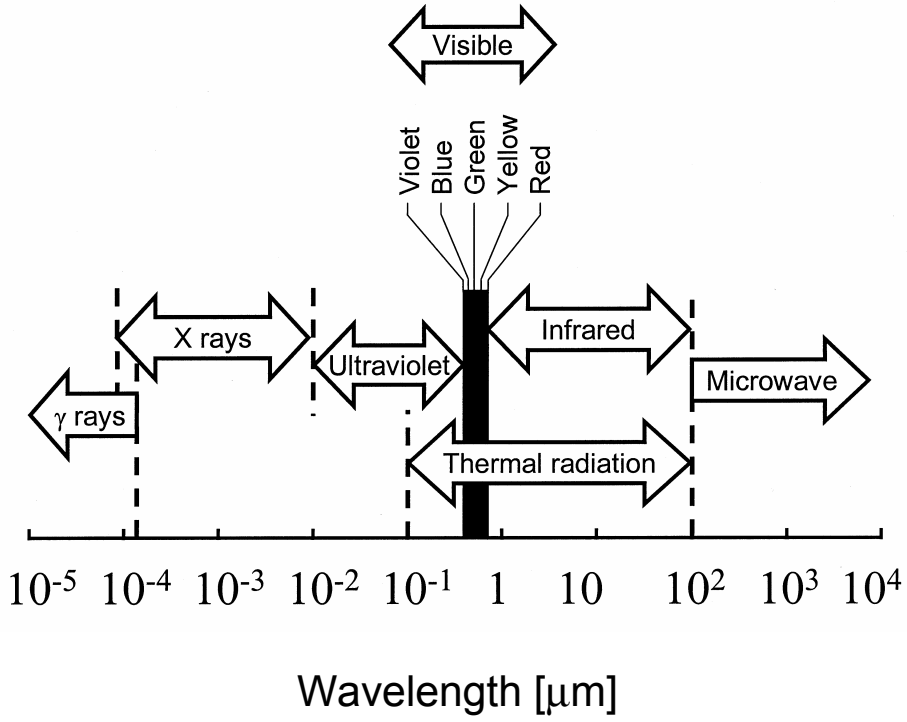

Fig. 1 – Electromagnetic spectrum (after: Rolle 2000).

Solar radiation

Solar radiation contains a wide range of wavelengths and fairly closely matches the emission of a black body at a temperature of about 5900K, but only parts of this range may penetrate the Earth's atmosphere (Fig. 2). For example, UV-radiation below 280nm does not reach the Earth's surface because shorter wavelengths are absorbed by ozone. Besides ozone other atmospheric molecules absorb parts of the solar radiation, for example molecular oxygen, water vapour, carbon dioxide and carbon monoxide as well as methane and nitrous oxides.

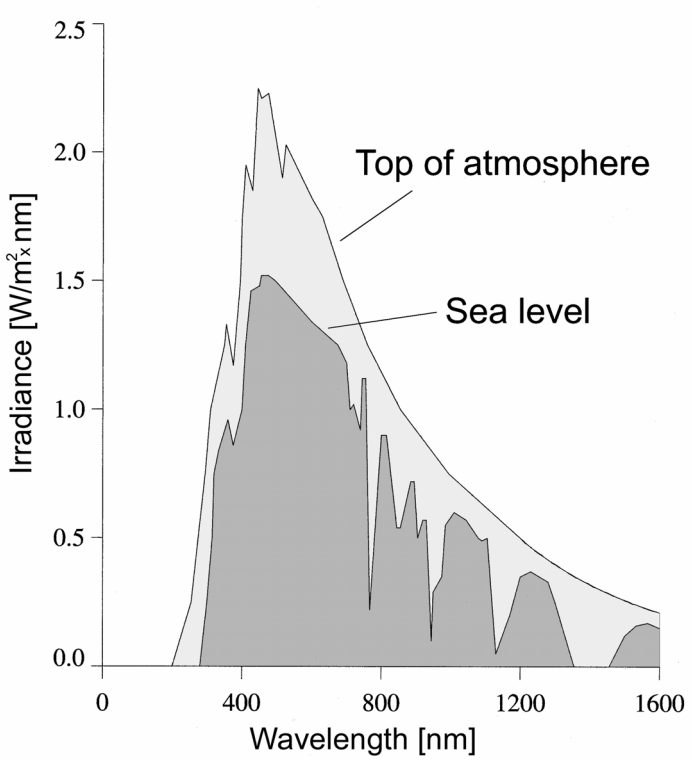

Fig. 2 – Irradiance spectrum of solar radiation at the top of the atmosphere and at sea level. Adapted after Andrews 2000. 'Irradiance' is the amount of electromagnetic energy incident on a surface per unit time per unit area.

About 10% of the Sun's emitted energy is at ultraviolet wavelengths, about 40% in the visible and ca. 50% in the infrared.

Thermal budget of caterpillars

Under natural conditions in the field the caterpillars' thermal budget will be influenced primarily by two factors: Solar radiation (and in the case of social caterpillars also radiation emitted by their tentmates) may be absorbed by the surface of the caterpillar and rise its body temperature. Simultaneously, the caterpillar will cool down by convection, mainly driven by the wind (Fig. 3).

For an individual caterpillar two parameters will predominantly influence its body temperature: The *color and nature of the integument* will influence the amount of radiation being absorbed and therefore determine the maximally achievable input of energy. In addition, *surface structures* may reduce convective heat loss (cf. Casey & Hegel 1981). To optimize this latter function, these surface structures themselves should only minimally affect radiant heat uptake.

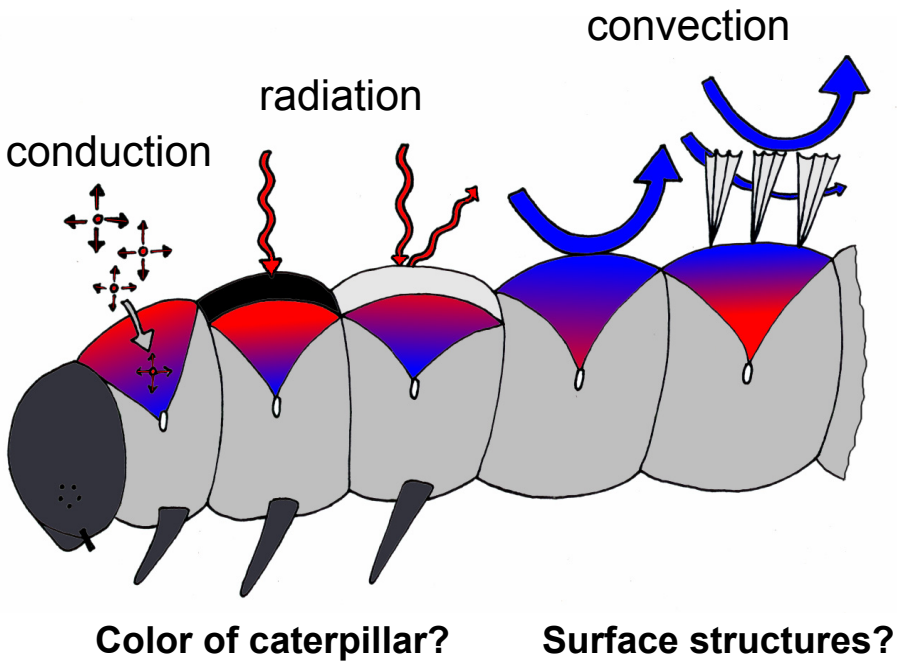

Fig. 3 – Schematized heat transfer modes influencing thermoregulation success in a single caterpillar. First segment: Influence of conduction (of minor importance). Second and third segment: Influence of color on absorbance: 'dark' surfaces (i.e. surfaces with high absorbance of short-wave radiation) maximize heat gain while 'pale' colors (i.e. surfaces that strongly reflect short-wave radiation) are not suitable for successful external heat gain. Fourth and fifth segment: Reduction of heat losses due to forced convection: Bare surfaces are strongly exposed to convection while surface structures (e.g. hairs) may minimize conductive heat losses.

Morphological characteristics of *Eriogaster lanestris* larvae relevant to thermoregulation

At the onset of my studies, I expected that successful thermoregulation is one of the most striking advantages promoting sociality in *Eriogaster lanestris* (cf. Ch. 1). Caterpillars only occur in sunny habitats and can be regularly seen being fully exposed to insolation. Thus, high irradiation is a natural part of the caterpillars' physical environment. In order to gain an assessment of the morphological characteristics for thermoregulation in *E. lanestris* it is necessary to quantify some thermal characteristics of its larval integument. Since about 50% of the sunlight reaching the Earth ranges from UV to the human-visible part of the spectrum, it is useful to measure the proportion of radiation reflected by the caterpillars integument.

For this purpose a full grown caterpillar was killed by freezing at –18°C. The hairs of the caterpillar were then carefully clipped with a fine pair of scissors and removed with a fine brush. Measurements were made using a reflectance spectroradiometer (Intraspec II diode-array photometer equipped with an MS125 spectrograph, Oriel, Stratford). This radiometer is capable of measuring a range from 300-690nm. Illuminating light was delivered from an XBO75W/2OFR xenon arc lamp (Osram, Munich). For a detailed description and technical specifications see Knüttel & Fiedler (2001).

Figure 4 shows that only about 3-4% between 300-600nm and less than 10% between 600-700nm is reflected by the caterpillar's body. Obviously, caterpillars of *Eriogaster lanestris* are highly capable of absorbing solar radiation. I did not measure the influence of the setae isolating the larvae, but results obtained by Casey and Hegel (1981) for gypsy moth caterpillars strongly suggest that the setae are very important for *E. lanestris* as well for reducing heat loss. It is noteworthy, that the long setae of *E. lanestris* are white or even transparent and should thus not influence radiant heat uptake by the caterpillars while basking in the sun.

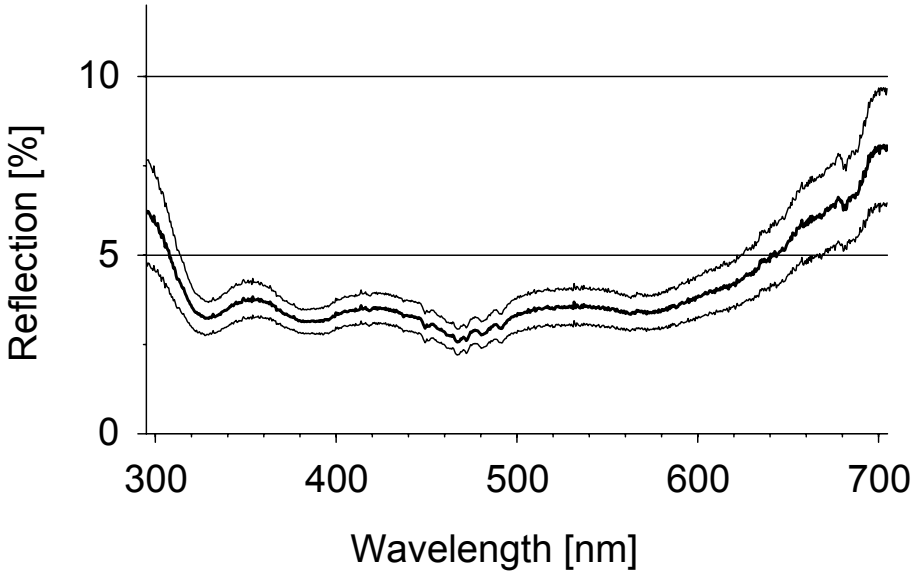

Fig. 4 – Reflection at different wavelengths of the integument of an *Eriogaster lanestris* caterpillar. 10 measurements at a shaved, dead caterpillar. Data shown: mean±1SD).

Social thermoregulation

It has repeatedly been shown that groups of caterpillars may obtain thermal gains high above that of solitary caterpillars (Porter 1982, Knapp & Casey 1986, Casey et al. 1988, Joos et al. 1988). The physical background of this observation is that there is an important effect of the mass of an object on its heat balance (cf. Fig. 5). By grouping, caterpillars multiply their effective body mass and therefore increase their maximum achievable heat gain. Although the precise form and slope of the relationship between body mass and thermal gains depends on a wide variety of climatic, behavioral, and physical parameters of the caterpillars' environment, the relative gain of large, dense larval groups as compared to individual caterpillars remains unaffected.

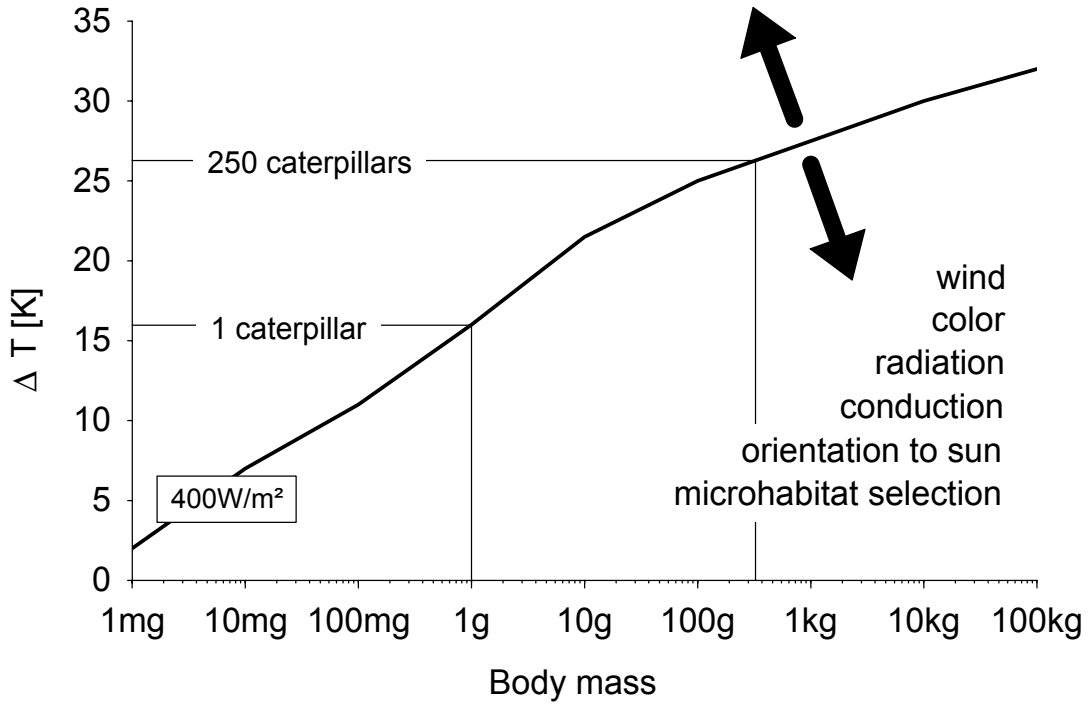

Fig. 5 – Effect of body mass on maximum thermal gains (ΔT= body temperature – air temperature) by basking. Indicated in the graph by thin lines are exemplary thermal gains of a single, nearly mature caterpillar (mass ≈1g) and of a cluster of 250 mature caterpillars (large, natural colony size. Grouping of caterpillars increases maximum potential temperature gain by 10K, which may double the velocity of physiological processes. Curve from Stevenson (1985) modeled for ectothermic animals at an irradiation of 400W/m² (=weak sunshine). Behavioral parameters (microhabitat selection, orientation to sun), environmental parameters (wind, radiation) as well as physical parameters of the substrate (conduction effects), and of the integument (absorption of short-wave radiation) may influence the slope of the curve considerably.

In other words, a small single caterpillar with low weight may not achieve body temperatures far in excess of ambient temperature even if it has good absorbing characteristics while the composed bodies of several hundred caterpillars may achieve body temperatures in the range of small ectothermic vertebrates such as lizards. As a consequence, this means that grouped caterpillars may maintain body temperatures that are optimal for digestion and development over many hours during a day whereas single caterpillars may only achieve optimum conditions during a small temporal phase of the day.

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Chapter 4

Thermoregulation by endogenous means: Metabolic heat production

Published as: Ruf, C.; Fiedler, K. (2000) Thermal gains through collective metabolic heat production in social caterpillars of *Eriogaster lanestris*. Naturwissenschaften 87, 193-196.

Abstract

We investigated thermal characteristics of aggregations of social, tent building caterpillars of the small eggar moth *Eriogaster lanestris* (Lepidoptera: Lasiocampidae). The highly synchronous behavior of individuals of the colony has important consequences for their thermal ecology. Air temperature in the tent fluctuates according to the caterpillars' activity: Air temperature slowly rises about 2.5-3K above the surroundings when caterpillars aggregate in the tent after feeding, and decreases rapidly when the larvae leave the tent. Thermal energy can be stored for a few hours when ambient temperature drops. Experiments show that metabolic heat production sufficiently explains this effect. As even minor additional heat gain may reduce developmental time, aggregating in the tent may thus confer selective advantages under overcast weather or at night, when behavioral thermoregulation through basking is not possible.

Introduction

As with any other ectothermic animals, caterpillars are normally not able to elevate their body temperatures above ambient temperature. Control of body temperature by physiological means has not been demonstrated and seems unlikely due to the small body size, homogeneous body composition or the lack of control of hemolymph flow to various body regions (Casey 1993). As a consequence body temperature tracks the surrounding temperature unless caterpillars regulate it by using external heat sources through behavioral

thermoregulation. Because of their small size and high surface-to-volume ratios, even basking caterpillars are usually able to rise their body temperature only a few degrees.

Caterpillars of the Palaearctic eggar moth *Eriogaster lanestris* (Lasiocampidae; for an account of the general biology of this species see Ebert 1994) are highly adapted to regulate their body temperature by basking. All instars have a black coloration that enhances radiant heating. Furthermore, they are thickly covered with setae that provide selective insulation and reduce convective heat exchange without affecting radiative heat gain (see Casey & Hegel 1981). However, the most striking feature of this moth species is the highly developed gregariousness of the larvae. These live together in large colonies of about 200 caterpillars or more (full siblings) and form dense aggregations when basking, which has been reported in other gregarious Lepidoptera species to maximize radiant heat uptake while reducing convective heat exchange by minimizing the relative body surface exposed (Joos et al. 1988). In addition, *E. lanestris* larvae spin a silk tent which serves as a home base, to which caterpillars return for digestion after each foraging bout. Such tents also function as a greenhouse resulting in an additional gain of heat (Carlberg 1980, Knapp & Casey 1986, Joos et al. 1988).

All earlier studies on the importance of social thermoregulation in caterpillars have concentrated on external heat sources (e.g. solar radiation), their effects on tent temperature and consequences for the thermal budget of the caterpillars. These investigations led to the conclusion that aggregations both in the laboratory and under field conditions never attain body temperatures significantly above ambient temperature when radiation is absent. Especially after dark the animals' temperature always dropped to ambient temperature. Although aggregating inside the tent reduces rates of individual heat loss, and some early studies suggested that clustered caterpillars generate enough metabolic heat to warm up the cluster above ambient temperature (e.g. Mosebach-Pukowski 1937), subsequent studies revealed no unequivocal evidence of an endothermic component to elevated body temperatures of gregarious caterpillars (Knapp & Casey 1986).

Only Breuer & Devkota (1990) observed that temperature inside the tent of pine processionary moths (*Thaumetopoea pityocampa*) was strongly tied to the presence or absence of the caterpillars, resulting in a slight increase (ca. 0.5-1.5K) in temperature when caterpillars return from their single foraging bout at night. The authors postulated that metabolic heat production is responsible for these findings, but no experiment was performed to rule out the possibility that the radiation of the illumination in the laboratory influenced the observed effect.

The aim of the present study was to test whether social caterpillars of *Eriogaster lanestris*, whose thermal ecology had never been studied before in detail, have any thermal advantage of aggregating in the tent when direct thermal radiation is weak or absolutely absent. As these caterpillars show several foraging bouts a day, it is also possible to investigate temperature effects during “day” and “night”. In addition, we investigated whether thermal differences between the tent and the surroundings can be caused by metabolic heat production only.

Material and Methods

For this purpose we kept colonies with last instar caterpillars in their original tents in an environmental cabinet under a 14:10h (L:D) light regime. Temperature changed between 20°C from 10:00 to 19:00 and 18°C during the rest of the day. For illumination we used five fluorescent tubes (Osram Lumilux DeLuxe: Biolux L58/72-965, 58 W each) which resulted in a maximum radiation of only 5-10W/m² (Sensor: LI-COR inc., QUANTUM Q 4557). For automatically monitoring the activity patterns of the colonies caterpillars had to pass a light barrier each time they left the tent for feeding. Temperature was measured to the nearest 0.1°C using high-precison thermal sensors (calibrated thermistor, HYGROTEC®, SEMI 833 ET). Temperature measurements and caterpillar counts were recorded and stored on a computer in one minute intervals.

Results of two experiments are presented here: In the first experiment four thermal sensors were attached to the tent: two of them inside the tent, one on its outer surface, and one a few centimeters away from the tent to measure

ambient temperature (T_a). Body temperature was therefore not measured explicitly for individual caterpillars, but as a group temperature of the aggregations (T_t). Data were taken continuously over a period of two days.

In a second experiment 30 last instar caterpillars were starved for a few hours and were then allowed to feed to repletion. Afterwards they were put in a small plastic box with airholes (volume: 250ml) which was lined with tissue paper. Four thermal sensors were attached inside the box (two on the bottom and one on each side of the box). As control a second plastic box was equipped equally, but no caterpillars were inserted. Each box was put into a larger polystyrene box and covered with acrylic glass to prevent that minor fluctuations of the surrounding temperature caused by the air condition would influence the measurements. Temperature was registered once per minute for two hours and the experiment was repeated three times. This experimental design ensured that any thermal differences between the two boxes were caused exclusively by heat produced by the caterpillars, but the effects of external radiation could be ruled out completely.

Results

Under the thermal and light conditions chosen in the first experiment colonies exhibited discrete bouts of activity separated by periods of rest. The behavior of individuals was strongly synchronized. Caterpillars left the tent three times a day, once every morning, every afternoon, and again around midnight (Fig. 1).

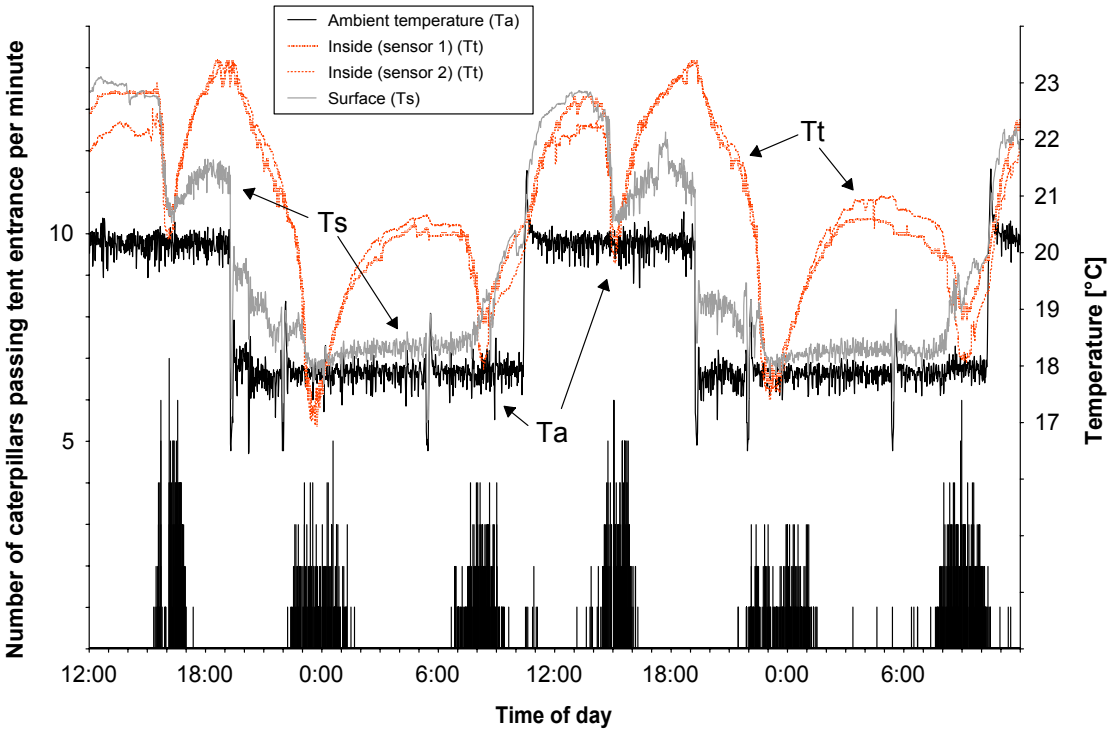

Fig. 1 – Dynamics of temperature changes in the tent in relation to foraging activity of an *Eriogaster lanestris* colony during the course of two days. Temperature in the tent is always higher than ambient temperature when caterpillars are in the tent (i.e. they are not active). Activity patterns are shown by the number of caterpillars counted per minute to pass the light barrier (left y-axis, bars), temperature by lines (right y-axis).

Activity patterns strongly affected temperature in the tent. When caterpillars were in the tent (i.e. they were not foraging) T_t was always higher than T_a, sometimes exceeding a thermal difference of 6K (Table 1). As soon as the caterpillars left the tent, T_t dropped immediately to T_a or even slightly below. The following increase in T_t coincided with the return of caterpillars after feeding.

Tent surface temperature was only slightly above T_a during the night, when all caterpillars usually rested in the tent, but it clearly increased in the morning when the animals usually aggregated and “basked” on the tent's surface. Measurements during the afternoon and evening were between these values, as only few caterpillars rested on the tent (Fig. 1).

There was no obvious difference in net heat gain inside the tent during day and night (mean thermal gain always ca. 2.5K; Table 1), leading to the conclusion that the low radiation of the fluorescent tubes did not affect our experiment.

Table 1. Differences between temperature measured in the tent and ambient temperature (in K, data from Fig. 1). Values are averaged over the course of two days; only measurements during the presence of the caterpillars are included; for calculating values during day and night phases of cooling and warming up in the environmental cabinet were disregarded.

Placement of sensor		All measurements outside foraging periods	During the day (T_a = 20°C)	During the night (T_a = 18°C)
	N	1825	615	652
Inside tent (sensor 1)	mean ± SD	2.7 ± 1.0	2.6 ± 0.3	2.4 ± 0.4
	maximum	6.7	3.7	4.0
Inside tent (sensor 2)	mean ± SD	2.7 ± 1.1	2.3 ± 0.5	2.7 ± 0.4
	maximum	6.8	3.7	4.5
Surface	mean ± SD	1.2 ± 0.8	1.9 ± 0.8	0.4 ± 0.1
	maximum	3.4	3.4	1.4

This conclusion is corroborated by temperature measurements of small groups of larvae in insulated boxes (Fig. 2). After the insertion of the caterpillars temperature in the box rose slowly, resembling the dynamics of temperature increase in the tent when caterpillars return from foraging. As effects of radiation can be excluded completely, this net heat gain must stem directly from metabolic heat production by the caterpillars. In our experimental setting metabolic heat production of 30 full-grown larvae was approximately 1.5K, compared to 2.5K in the much larger natural aggregations in the original silk tent.

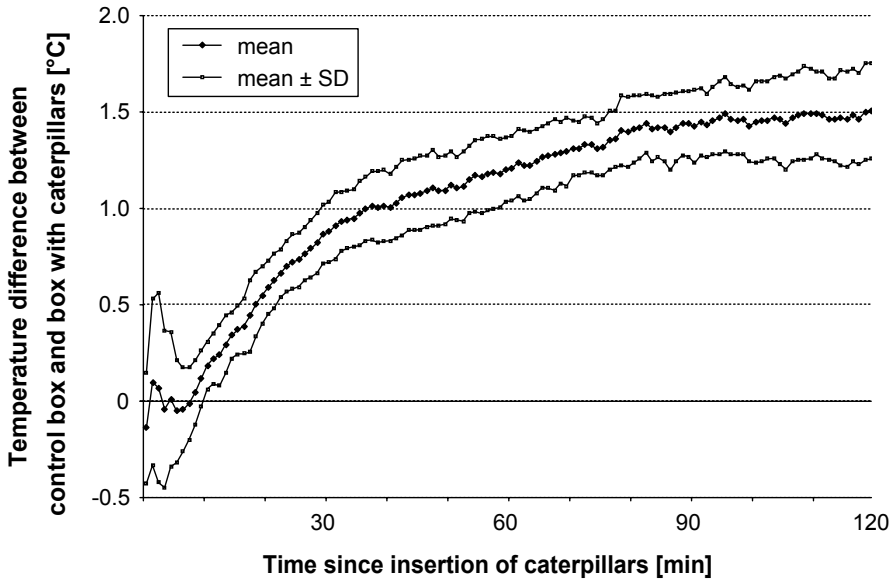

Fig. 2 – Changes in temperature in a plastic box after insertion of 30 fed last-instar caterpillars. Temperature differences refer to the control box without caterpillars. Measurements are averaged for all four sensors and three replications of the experiment.

Discussion

Metabolic processes always produce heat as a by-product, but caterpillars have no adaptations for storing or even regulating it and heat production of a single caterpillar is very small.

The maximum temperature a body can achieve is a function of its mass (Stevenson 1985). By aggregating larvae multiply their effective mass, heat production of all group members is added, and body temperature of every individual may increase much more than it would ever be possible for a single larva. Furthermore, the sphere-like arrangement of aggregations reduces heat exchange with the surroundings by minimizing the volume to surface ratio. Even when caterpillars rest on the tent in the morning, aggregating is advantageous because the tent provides a large boundary layer that reduces convective heat loss (Joos et al. 1988).

It has often been emphasized that the tents of social caterpillars do not provide any insulation (Carlberg 1980, Knapp & Casey 1986). Nevertheless, as can be seen in Figure 1, cooling is clearly delayed after the fall in T_a during evenings,

and small fluctuations of T_a do not affect T_t at all. The thin silk layers themselves are probably of minor importance, while the architecture of the tent and the caterpillars' bodies contribute most to overall heat storage. Tents of *E. lanestris* are constructed in multiple silk layers with air spaces between them. Heat produced by the caterpillars may therefore be reflected between the layers without being emitted (greenhouse effect). The spaces between the layers probably have a strong insulating effect as the thermal conductivity of air is very low. Furthermore, the dense aggregation of caterpillars itself serves as heat accumulator since both solids and liquids have a higher thermal capacity than the surrounding air. All these effects reduce thermal losses through convection.

The observation that T_t is always about 2.5K higher than T_a, independently of the absolute value of T_a (see Table 1), emphasizes the overall importance of T_a in affecting T_t. However, although caterpillars cannot regulate their body temperature by metabolic heat production independently of T_a, they may elevate at least it by collective warming. Dense aggregation in the tent therefore allows the caterpillars to build up their own microclimate even when external heat sources are absent.

Caterpillars of *E. lanestris* hatch in early spring, when temperatures are often below a physiological threshold suitable for growth. Even small increments of temperature excess may cause large reductions in developmental time (Rawlins & Lederhouse 1981, Scriber & Lederhouse 1983, Schroeder & Lawson 1992) and therefore have important consequences on larval survival and individual fitness. Lance et al. (1987) demonstrated that in gypsy moth caterpillars, which are comparable in size to *E. lanestris*, an increase of only 2K (i.e. comparable to the thermal gain shown above) during daytime leads to a reduction in developmental time by more than one week (13%).

When developmental time is shortened, larvae decrease their exposure to natural enemies. In early spring species a fast development may further maximize the intake of high quality food, which is rich in nitrogen and water and available for only a short time after budbreak (Parry et al. 1998).

Living gregariously involves great risks, such as conspicuousness to natural enemies and resource shortages through intracolonial competition for food. Especially costs associated with behavioral thermoregulation (e.g. exposure to predators and parasitoids when basking) must be offset by advantages such as an increased rate of food intake and growth rate.

Multiple selective advantages may favor the evolution of social life-styles in caterpillars, such as the improved effectiveness of aposematic colors (Sillén-Tullberg 1988), facilitated feeding by overcoming plant defense (Clark & Faeth 1997), and group defense against enemies (Lawrence 1990). In *E. lanestris* thermal ecology should be of special importance as larvae encounter thermal conditions that are often unsuitable for growth, suggesting an improved heat balance to be particularly critical. *E. lanestris* larvae utilize both solar radiation through basking and mutual warming through metabolic heat production and thus exhibit the most advanced strategy of obtaining thermal gains known so far from any Lepidopteran species with social caterpillars.

High synchronization of individuals in social caterpillars during activity periods facilitates foraging through cooperative search for food (Fitzgerald & Peterson 1988) and is essential for building the silken tent (Fitzgerald & Willer 1983). In addition, as demonstrated above, thermoregulation by basking and warming by metabolic heat production are beneficial only if caterpillars rest in the tent simultaneously, providing each other insulation and warmth.

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Chapter 5

Tent-based thermoregulation: Behavioral mechanisms and physical features of the tent

Published as: Ruf, C.; Fiedler, K. (2002) Tent-based thermoregulation in social caterpillars of *Eriogaster lanestris* (Lep., Lasiocampidae): Behavioral mechanisms and physical features of the tent. Journal of Thermal Biology 27, 493-501.

Abstract

1. We investigated thermoregulatory behavior of social tent building caterpillars of *Eriogaster lanestris.*

2. The silk layers of the tent shield most of the incoming radiation and reduce heat exchange with the surroundings by convective heat loss.

3. As a consequence its interior provides a wide range of temperatures.

4. By changing their position inside or on the tent caterpillars are able to stabilize their body temperatures at 30-35°C over a wide range of ambient temperatures as long as solar irradiation is sufficiently strong.

5. Overall behavioral thermoregulation takes precedence over the tent's physical features.

Introduction

Thermal conditions influence growth and activity patterns of insects in all stages of their lives. In contrast to some winged insects that are able to produce warmth by endogenous means (i.e. shivering of flight muscles, Heinrich 1981) most ectothermic insects like caterpillars are usually closely linked to their thermal environment. Although caterpillars may be able to elevate their body temperatures by metabolic heat production (Mosebach-Pukowski 1937, Breuer & Devkota 1990, Ruf & Fiedler 2000) temperature gains are only weak and caterpillars may have to elevate their body temperatures additionally by basking in the sun. Thus, caterpillars which require a specific temperature range must

thermoregulate behaviorally by various means to overcome developmental threshold temperature. The most important way of behavioral thermoregulation is to change their orientation and/or position in the habitat towards incoming radiation (for an overview see: Stevenson 1985b), especially under cold weather conditions.

Solitary caterpillars may only attain low gains of temperature by basking due to their small body mass (cf. Rawlins & Lederhouse 1981, Karban 1998). In contrast, many social caterpillars are especially adapted for effectively gaining higher body temperatures by building dense aggregations that are known to reduce convective heat losses (Joos et al. 1988). Furthermore, they enhance the maximum achievable heat gain by increasing their effective body mass (Stevenson 1985a). It has repeatedly been shown that social caterpillars may rise their body temperature far above ambient temperature by basking and that they are able to maintain high body temperatures throughout a wide range of ambient temperatures (Porter 1982, Knapp & Casey 1986, Casey et al. 1988, Bryant et al. 2000). Less attention has been drawn to the fact that in particular social caterpillars may suffer from excessive solar radiation and have a severe risk of overheating even in temperate zone regions. Thus, avoidance of overheating under high solar irradiation should be a central feature of thermoregulation in social caterpillars in much the same manner as maximizing body temperature on cold days (cf. Seymour 1974, Capinera et al. 1980, Frears et al. 1997).

Only few lepidopteran species with social caterpillars have evolved a stationary resting and molting site that is used as a home-base throughout the social phase of the larval development. Even fewer species build a dense silken three-dimensional tent with an accessible interior (cf. Fitzgerald 1995, Costa & Pierce 1997). Communal spinning at regular intervals results in a multi-layered structure of the tent due to shrinkage of the silk shortly after deposition (Fitzgerald & Willer 1983). The layers are separated by air-filled gaps within which the larvae rest.

The role of the tent in behavioral thermoregulation has been the object of several studies in social caterpillars (Joos et al. 1988, Breuer & Devkota 1989, Fitzgerald & Underwood 2000). It was suggested that the tent functions somewhat like a greenhouse, heated by trapping solar radiation but little affected by external convection. However, the physical features of the tent have never been analyzed and there is no information about features of the silk layers in relation to irradiation which must be responsible for the observed effects.

Caterpillars of the small eggar moth *Eriogaster lanestris* (Lepidoptera: Lasiocampidae) hatch in early spring shortly after budbreak of their hostplants (i.e. blackthorn, *Prunus spinosa*, and other deciduous trees and shrubs, Ebert 1994). The totally black caterpillars typically face central European spring and early summer weather with frequent spells of low temperature but often high radiation. Caterpillars build a dense silken tent which is continually extended up to the end of the penultimate instar. In nature, larvae pupate in June to early July. Since tents are usually formed on sun-exposed hedgerows or hostplant trees, caterpillars are expected to experience also periods of occasional heat, in particular towards the end of their feeding period. Thus, *E. lanestris* should be particularly prone to both aspects of thermoregulation in social caterpillars, i.e. elevation of body temperature early in the season and the risk of overheating later on.

The aim of the present study is to analyze the thermoregulatory behavior of *E. lanestris* under field conditions. Particular attention is paid to the interrelation between behavior, microclimate in the tent, and thermoregulation.

Material and Methods

Animals: Whole colonies with caterpillars of *Eriogaster lanestris* were collected in the field and fed with leaves of blackthorn. Resulting cocoons were over-wintered in a refrigerator with interior lighting (5°C, 8:16h L:D). Moths hatched within a few hours after the cocoons were warmed up in spring and were put in a plastic box (10 liter volume) with blackthorn twigs for mating and egg

deposition. Whole egg masses with newly hatched caterpillars were placed on small bunches of blackthorn twigs until small tents had been established. Some of the tents were cut out of the bunches and transferred to wooden frames (cf. Fitzgerald 1980). Colonies were kept in an environmental cabinet (20°C, 14:10h L:D) until the end of the second instar and were then either transferred to natural hostplants (*Prunus* sp., *Betula* sp.) in the Botanical Garden of the University of Bayreuth, or to the roofdeck of the university building, where the tents were connected to bunches of the host-plants by a plastic rod. Tents on the roof were protected from birds by a coarse meshed cage (2.5×2.5×1.5m, width of meshes ca. 2cm) that did not influence solar radiation. To these colonies fresh food was provided daily in ample supply. For measurements of individual body temperatures a total of 17 colonies (12 on the roofdeck, 5 in the Botanical Garden) were used for data acquisition and data were pooled.

Temperature and radiation measurements: Temperature measurements of individual caterpillars were only carried out during the late fourth and early fifth instar to level out effects of caterpillar size and weight.

Body temperature (T_b) of individual caterpillars was measured to the nearest 0.1°C by cautiously pressing a miniature coated thermocouple (NiCr / Ni, ca. 0.1mm diameter coated with Inconel 600; TMG, Martinroda, Germany) which was attached to a laboratory thermometer (DTM 3010) onto the back of the caterpillar for about 3s to allow the reading to stabilize. Knapp and Casey (1986) reported no significant difference between this non-invasive method and alternatively piercing the cuticle of larvae to directly measure internal T_b. In addition to body temperatures of caterpillars temperature was measured immediately afterwards at different sites in and at the tent, namely the temperature under the sun-facing surface of the tent (T_{surf}), temperature inside the tent in a central position (T_{ins}), temperature in the shaded part of the tent opposite to the irradiated side (T_{sh}), and ambient temperature in the shade close to the tent (T_a). For one analysis we calculated maximum temperature inside the tent by choosing the greatest value of either T_{surf}, T_{ins}, or T_{sh} which we call $T_{tent,max}$.

Data for global radiation were provided by a meteorological station in the Botanical Garden in Bayreuth at about 300m linear distance from the study site.

Long-term measurements: Temperature progression in the tent was long-time monitored with calibrated thermistors (Hygrotec: SEMI 833 ET) connected to a computer. T_{surf}, T_{ins}, T_{sh}, and T_a were automatically recorded to the nearest 0.1°C in one minute intervals. Four colonies with about 200 caterpillars each were monitored over their entire fourth instar. In addition, activity of caterpillars was recorded continuously with light barriers (cf. Ruf & Fiedler 2002).

Caterpillars' position: Caterpillars often changed their positions in and on the tent while resting between foraging periods. Position was classified in three categories which are either 'in the sun' (i.e. outside the tent on its irradiated surface), or 'inside the tent', or 'in the shade' (i.e. outside the tent on its non-irradiated side or on branches nearby the tent), respectively. The exact position of caterpillars in the tent could not be analyzed due to the density of the silk that did not allow for detailed observations.

Spectral photometry and transmittance of radiation: To investigate physical features of the tent the utmost silk layer of a tent of a laboratory colony was cautiously detached from the wooden frame and spread on a smooth surface. Impurities of the silk e.g. larval faeces were not removed because of the danger of damaging its structure. Moreover, such impurities accumulate over the development of each larval colony and thus are part of their natural conditions. Spectral transmission of six samples of differently dense silk layers was measured in the spectral range from 190-1100nm with a spectrometer (Perkin Elmer, Lambda 2, UV-Vis with adjusted deuterium lamp (190-327nm) and tungsten-halogen lamp (327-1100nm)). Although this range did not cover the whole spectrum emitted by the sun and passing the earth's atmosphere, it covers at least the most energy rich part of natural sunlight.

In addition, a piece of silk was mantled over the sensors of a radiometer (type CRN1, Kipp & Zonen, NL; sensor for short wave radiation, λ=300-3000nm, thus covering the total range of the sunlight). The instrument prepared in this way was attached to a meteorological mast to analyze the portion of solar radiation

permeating the silk layer. Data were saved by a data logger in 10min intervals over the course of five days. Data were then compared to the data obtained by a non-covered identical sensor installed at the meteorological station.

Control for measurement errors / statistics: To assess the sensitivity of our thermistors to radiation errors we installed one thermistor and a special sensor whose measurements are nearly free of radiation errors (type Pt150 (12μm platinum wire), AIR, Boulder, CO) in an empty caterpillar tent near the meteorological station. Data from both thermal sensors were saved by a data logger for five consecutive days with high solar radiation in July in 10min intervals. We calculated standardized residual values from regressing thermistor on Pt150 data. Since the thermistor showed an exponential characteristic, data of the Pt150 sensor were log-transformed prior to regression analysis. Residual values were then correlated with radiation intensity to assess whether the thermistor used for longtime measurements had higher radiation errors at increasing levels of solar irradiation.

All statistical analyses were conducted using the STATISTICA software package (StatSoft, 1999) or were calculated according to Sachs (1992).

Results

Reliability of thermistor measurements: Temperature measurements of the thermistor SEMI 833 ET and the sensor Pt150 were highly significantly correlated (R^2=0.97, $p<0.001$, N=288). Standardized residual values were positively correlated with radiation ($r=0.22$, $p<0.001$) but data scattered widely and linear regression of residuals on radiation only explained 5% of the variance observed (R^2=0.05). Thus, radiation errors of the thermistors were assessed to be in a negligible range for our experiments.

Temperature in the tent: Temperature in the tents inhabited by caterpillars varied widely over the course of a day (Fig. 1). On sunny days temperature in the tent rapidly exceeded ambient temperature after sunrise, reaching maximum values of more than 50°C. During dusk, temperature in the tent rapidly equated to or even fell slightly below ambient temperature.

Within the tent there was a steep temperature gradient according to the position of the sensors. Until noon temperature was highest just below the surface of the tent, medium inside the tent, and lowest at the non-irradiated side of the tent. As expected, this order changed when the sun changed its position (cf. Fig. 1, upper graph). On days with overcast weather or rainfall differences were less pronounced (Fig. 1, middle and lower graph). Temperature gains of the tent compared to ambient temperature were significantly correlated with solar radiation (R^2=0.94; Fig. 2) with only low temperature gains on clouded days and maximum gains of about 25K on sunny days.

In contrast to these findings in tents with caterpillars, temperatures measured within the empty tent were always within 2-5K compared to T_a even on sunny days (N=360 measurements during five days, 0900 - 1900h).

Caterpillars' behavior / body temperature: Caterpillars changed their position on or in the tent throughout the day. Even though single caterpillars occasionally behaved differently, the majority of siblings behaved as a unit, resting either on the irradiated surface of the tent, where they formed a dense aggregation with orientation to the sun, or in the tent. When ambient temperatures increased towards noon and early afternoon on sunny days aggregations first became loose, then caterpillars crawled on the underside of the tent apart from the incoming radiation, or finally even left the tent to rest on the underside of branches near the tent. Even though it was impossible to unequivocally assign the caterpillars' choice of position to a given temperature range the frequency of occurrence of either positions at different temperatures was significantly different (Fig. 3).

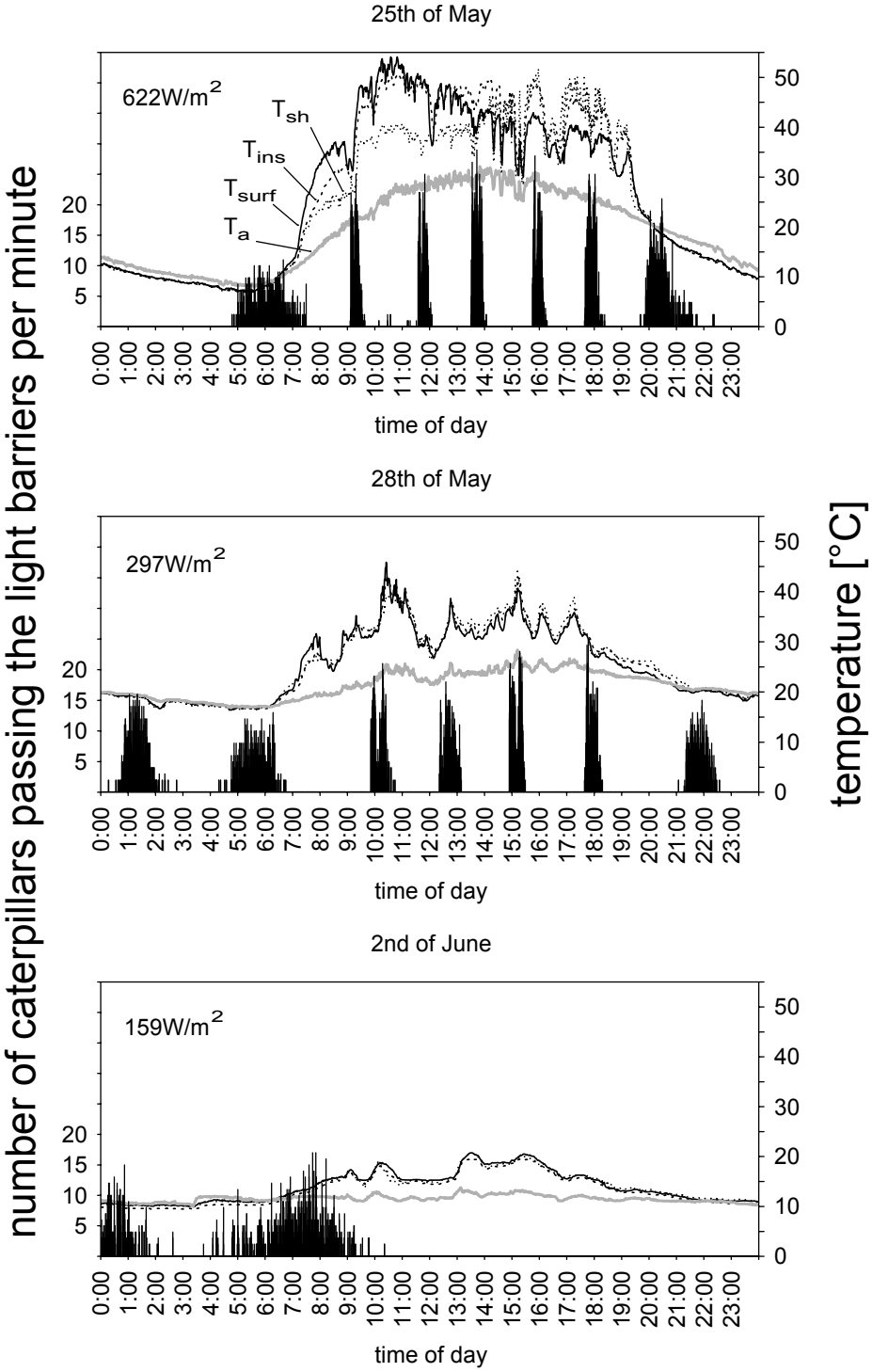

Fig. 1 – Exemplar chart recordings of temperature progression (lines) in the tent and activity patterns of the caterpillars (bars) on three days with different weather conditions. Average radiation (0700-1900h) per day is shown in each graph. On June, 2nd rain fell continuously from 0800h onwards. Sunrise: ca. 0500h, sunset: ca. 2100h. Grey line: ambient temperature (T_a). Black lines: solid: T under tent surface (T_{surf}), dashed: T inside tent (T_{ins}), dotted: T at shaded side of tent (T_{sh}).

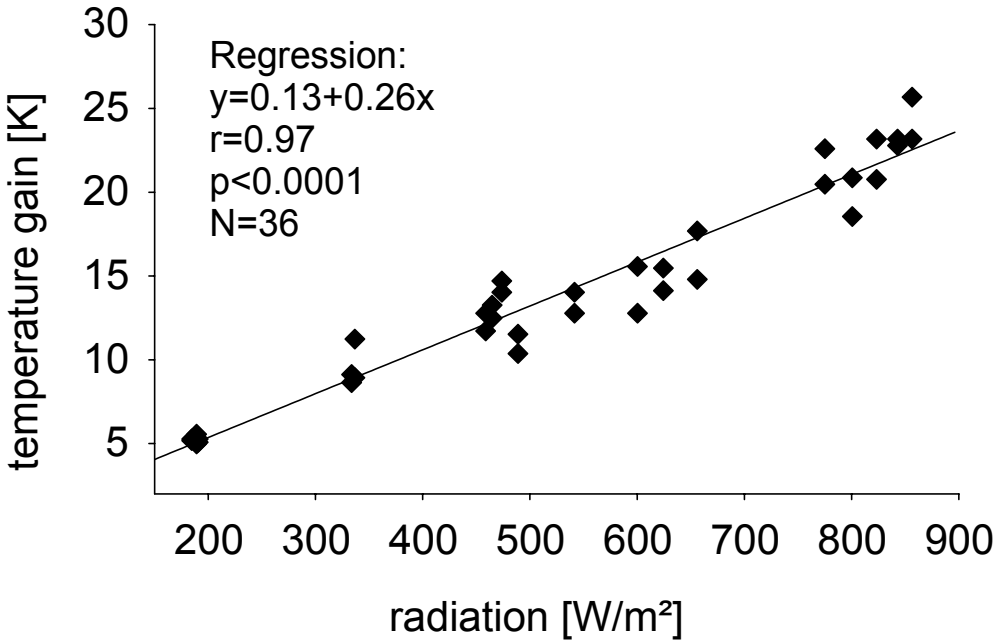

Fig. 2 – Temperature gain of tents inhabited by caterpillars depending on solar radiation. Pooled data of four colonies (36 colony days). Values for temperature and radiation are averaged over a period of 6 hours (3 hours before and after zenith of the sun). Temperature values averaged over maximum values measured by any of the three sensors placed in the tent.

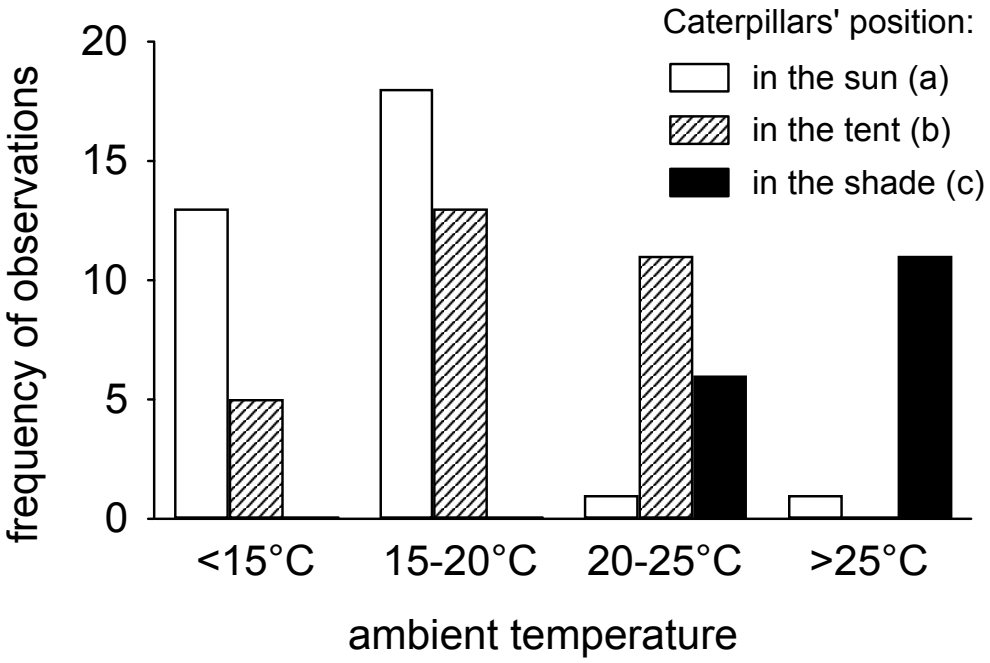

Fig. 3 – Ambient temperature and position of caterpillars relative to the tent during the day. The observed frequency distributions of all categories are significantly different from each other (all combinations significant at $p<0.001$ after sequential Bonferroni correction, generalized Fisher's exact test. a-b: $F_i=13.71$, a-c: $F_i=43.97$, b-c: $F_i=30.81$).

Threshold temperatures for the change from one position to another (here defined as the temperature of which the regression model predicts 0.5 probability of behavioral change) were estimated by logistic regression: Change from the sun exposed side of the tent to its interior (and vice versa) occurred quite gradually with a threshold ambient temperature of 19.3°C (goodness of fit of logistic model: χ^2=8.87, p<0.003), while the change from the interior to the shaded side of the tent occurred much more rapidly at 23.3°C (χ^2=42.09, p<0.0001).

Body temperatures of caterpillars were always elevated compared to ambient temperature during the day. During sunny weather caterpillars achieved highest temperature gains in the early morning when temperatures were low but radiation was already strong. In contrast, temperature gains were smaller relative to ambient temperature around noon when T_a was high (Fig. 4 above). This significantly negative relationship between T_a and (T_b-T_a) is explained by the caterpillars' behavior (Fig. 3): with increasing temperature *E. lanestris* larvae seek shadow and avoid direct solar radiation. Thus, caterpillars were able to maintain body temperatures of about 30-35°C over a wide range of ambient temperatures (Fig. 5).

Under overcast weather conditions temperature gains were smaller, and increased with increasing ambient temperature (Fig. 4, lower graph). Under such conditions sun-avoidance behavior was never observed in the caterpillars.

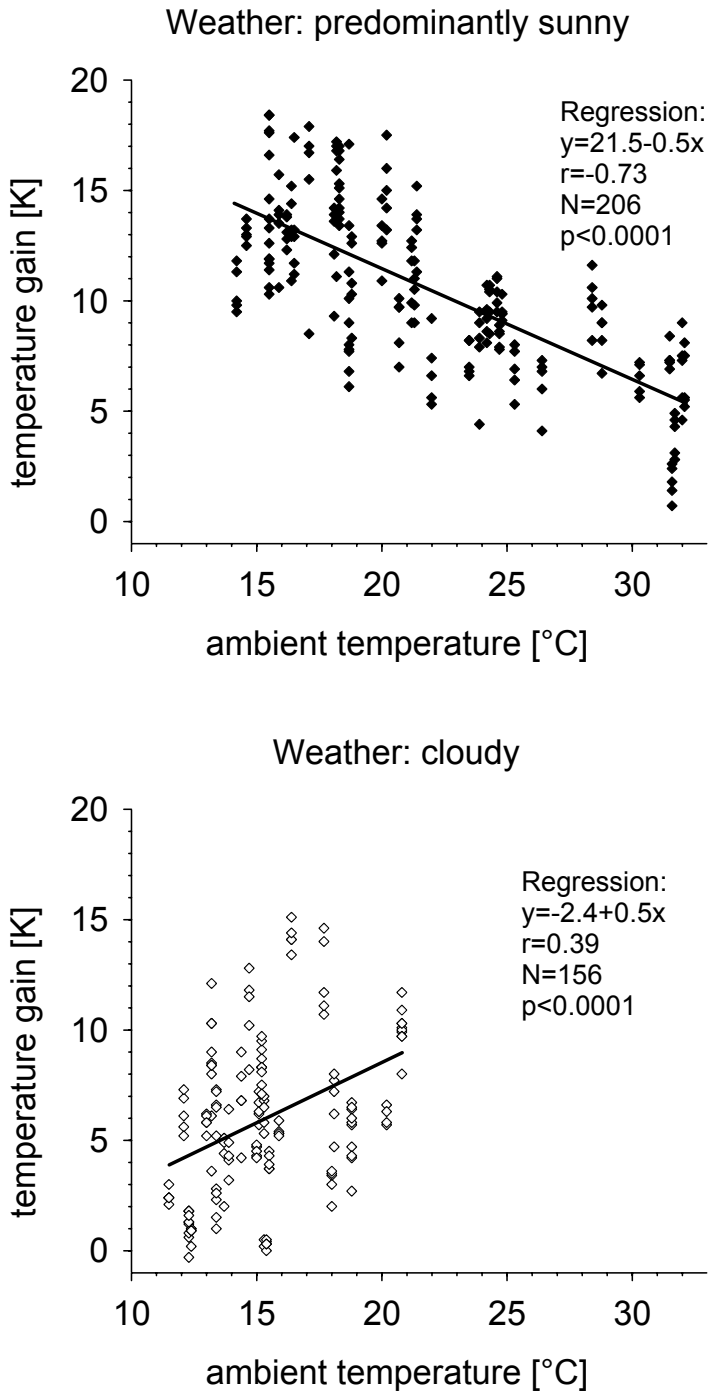

Fig. 4 – Temperature gain of individual caterpillars at different ambient temperatures. Upper graph: data gained at predominantly sunny weather conditions (radiation: 966±285W/m^2). Graph below: data gained during overcast weather (radiation: 387±238W/m^2).

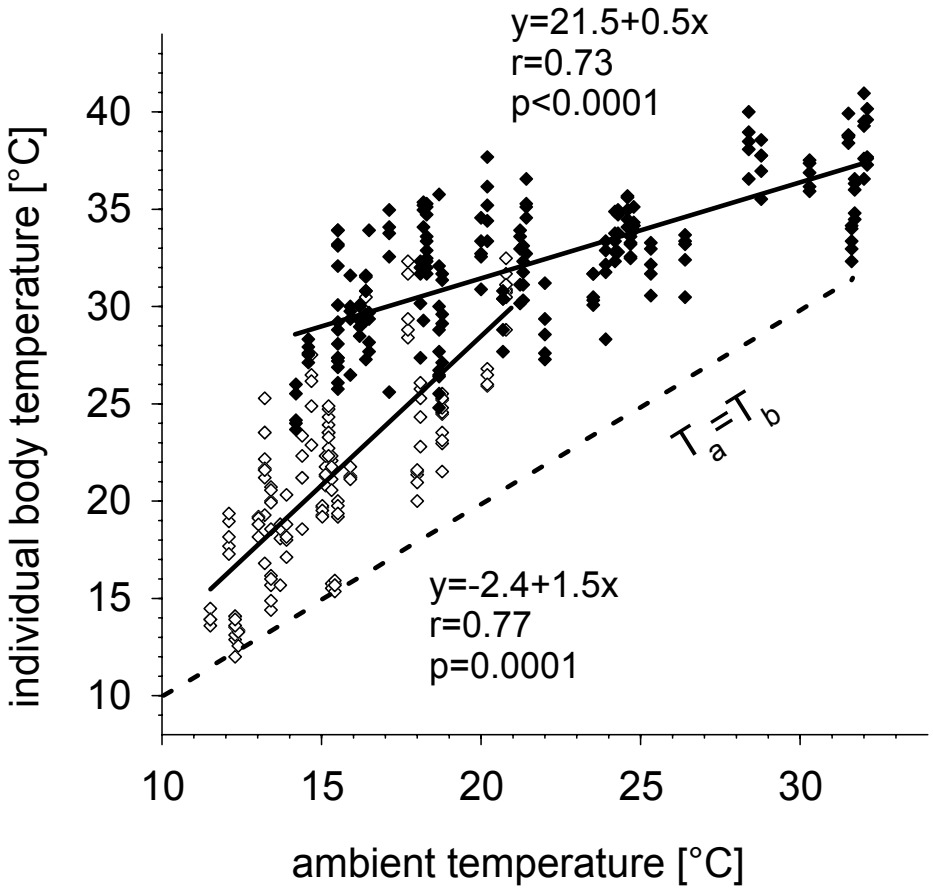

Fig. 5 – Individual body temperatures of caterpillars at different ambient temperatures during cloudy (white diamonds) and predominantly sunny (black diamonds) weather conditions. Slopes of regressions are significantly different (t_{357df}=10.8, p<0.0001) and boths functions are significantly different from T_a=T_b (cloudy: t_{154df}=5.3, p<0.0001; sunny: t_{203df}=15.3, p<0.0001).

Caterpillars regulated their body temperature behaviorally by seeking the optimal microclimate in and around the tent (Fig. 6). Up to a $T_{tent,max}$ of ca. 30°C, the caterpillars' body temperature closely followed the temperature of the warmest part of the tent. When T_b exceeded about 35°C the caterpillars ceased to aggregate and rested individually in the shade, either on the underside of the tent or in the nearby vegetation. Thus, T_b was decoupled from tent temperature at high T_a and approximated a satiation value of 38°C (value determined by regression analysis, Fig. 6). Under extreme conditions the caterpillars hang from the shaded side of the tent, only being fixed by their abdominal prolegs, to maximize convective heat loss. None of the caterpillars ever died on the tent because of overheating, but caterpillars which fell from the tent and got stuck to a glue strip died within a few minutes.

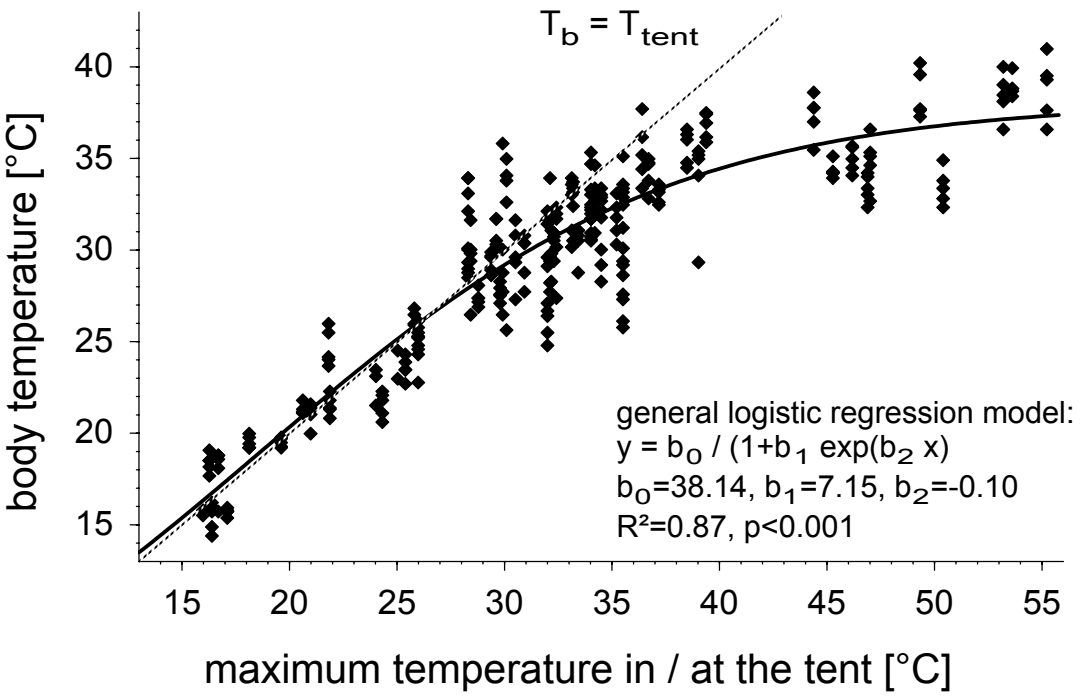

Fig. 6 – Influence of behavior on individual body temperature: Body temperature follows maximum temperature in the tent up to 30°C. At higher temperatures caterpillars prevent overheating by disbanding aggregations and leaving the tent.

Physical features of the silk layers:

Spectral photometry showed that transmission remains rather constant from 190-1100nm with a slight decrease of transmission towards the near IR-range of the spectrum (Fig. 7). Radiation did not easily penetrate the silk layers. Even the thinnest layer measured showed only a mean transmittance of 63%, and less than 4% of incoming light was transmitted through the thickest layer. Since the tent consists of multiple silk layers almost all short-wave radiation from sunlight is blocked.

When the radiometer sensor (λ=0.3-3μm) was covered with silk, only about 50% of the total solar energy simultaneously measured by the non-covered instrument permeated through to the sensors surface (mean$\pm$SD=51.6$\pm$19.6%, pooled data from 360 measurement intervals spread over five days from 0700-1900h CEST).

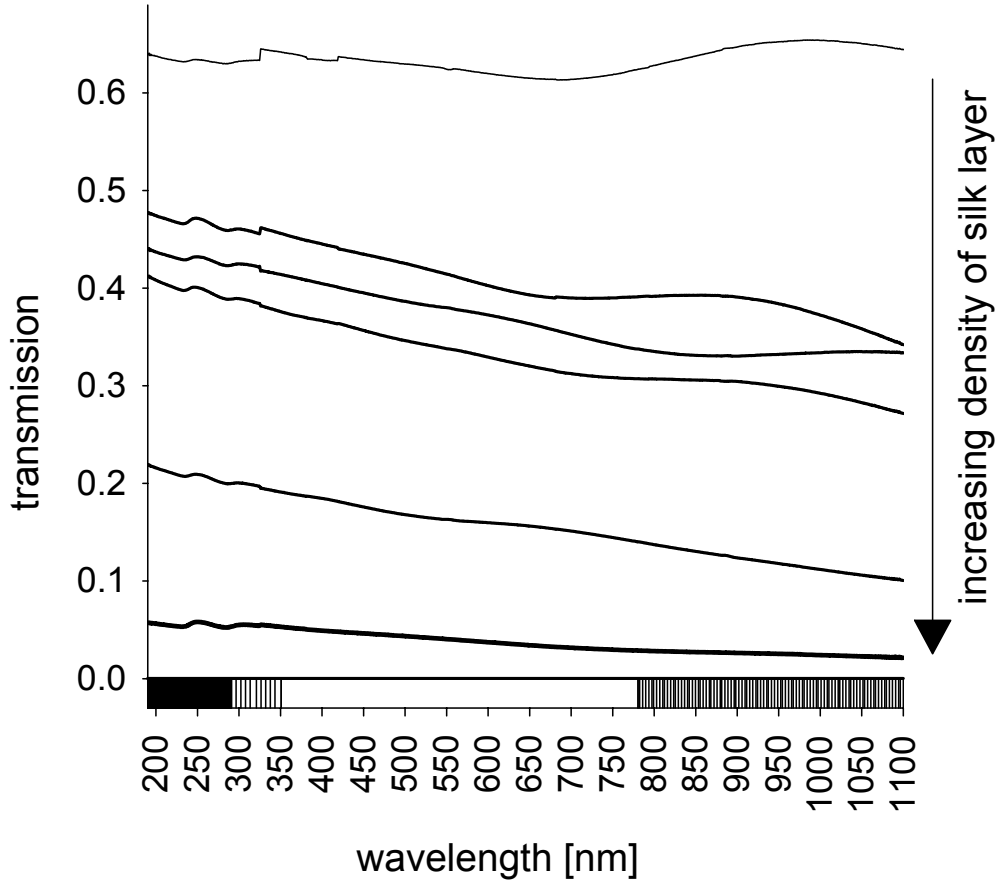

Fig. 7 – Spectral transmission of six *Eriogaster lanestris* silk layers of different thickness. Different bars at the bottom of the graph show the visible range of the light (white), ultraviolet light (dashed), near-infrared (tightly dashed) and the proportion of the solar spectrum which is not transmitted through the earth's atmosphere under natural conditions (black).

Discussion

Our results show that caterpillars of *Eriogaster lanestris* are typical thermoregulators which are able to maintain high and relatively constant body temperatures over a wide range of ambient temperatures. Thermoregulation is improved considerably by the tent, a multi-layered silk texture which creates a heterogeneous microclimate. In *E. lanestris*, the effectiveness of thermoregulation does not only depend on the incoming solar radiation, but is likewise mediated by the caterpillars' behavior and the physical properties of their tent.

At the onset of their development, caterpillars of *E. lanestris* are active at a time of year when the average daily ambient temperature is often far below the optimum temperature required for growth and development. However, univoltine early spring larvae are frequently under great time pressure to complete their life cycles because availability of high quality larval resources is restricted to a short period of the year (cf. Parry et al. 1998). It is well known that leaf quality is best shortly after budbreak and that nutrient as well as water content rapidly decrease during the following weeks (Hunter & Lechowicz 1992, Slansky 1993). Caterpillars of *E. lanestris* failed to grow if they hatched about eight weeks after budbreak (C. Ruf, unpubl. obs.).

In insect larvae increased body temperatures raise rates of digestion and growth (Rawlins & Lederhouse 1981, Knapp & Casey 1986, Fischer & Fiedler 2001) and thereby shorten development time. However, most social caterpillars restrict foraging to the night or special daytimes in order to avoid exposure to natural enemies. Thus, they might not be able to benefit from increased body temperatures. In contrast, caterpillars of *Eriogaster lanestris* show plastic foraging patterns that are strongly influenced by temperature (Ruf & Fiedler 2002). If temperatures are high, caterpillars are able to process food quickly and perform more foraging bouts per day than on cool days. Consequently, effective thermoregulation in this species should play a central role in enhancing food intake and growth.

It proved difficult to explicitly describe the position of the caterpillars in or on the tent solely on the basis of temperature. Obviously, additional, non-tested parameters including factors other than thermal constraints, influence decision-making of caterpillars. As a rule, however, caterpillars rested on the surface of the tent during the cold phases of a day, when radiation penetrating the tent possibly was too low to effectively warm the caterpillars (i.e. in the early morning or during phases of sunshine after rain). At higher temperatures, but also during phases of rainfall and in the absence of radiation at night, caterpillars made use of the multiple advantages of the tent like reduced convective heat loss, optimized surface-to-volume ratio, and protection from natural enemies, and retreated into the tent. When radiation was even higher,

temperatures in the tent rapidly exceeded physiological tolerable temperatures (>30-35°C) and caterpillars left the tent to rest in the shade. Laboratory studies with different arthropod species showed that developmental rates are often maximal at about 30°C but rapidly decrease at temperatures above 35°C (Logan et al. 1976, Taylor 1981, Lactin et al. 1995, Kingsolver 2000). Only few species of soft-bodied caterpillars are known to survive at temperatures slightly higher than 40°C at least for limited periods of time (Casey 1976). However, aggregation behavior has been shown to reduce water losses under high heat loads in a tropical saturniid caterpillar (Klok & Chown 1999).

The tent facilitates a three-dimensional clumped formation of the caterpillars that are thus able to form a composite body with an optimal volume-to-surface ratio that minimizes convective heat loss. Full grown larvae of *E. lanestris* that still form a social unit achieve body weights of about 1.3±0.1g (mean±SD, N=20) resulting in an effective body mass of ca. 260g for an average colony that comprises 200 caterpillars. Simulations suggest that such an increase in body mass may double the maximum temperature gain achievable by basking (Stevenson 1985a,b).

Other experimental studies showed that aggregations of caterpillars maintain body temperatures far in excess of single caterpillars of the same size independent of their orientation to the sun (Joos et al. 1988, Klok & Chown 1999) and that large groups achieve higher temperature gains than small groups (Halperin 1990). In addition to thermal benefits group basking serves to keep the entire colony at about the same T_b, thereby facilitating synchronous growth of the colony despite low T_a and high thermal sensitivity of growth.

Under laboratory conditions, in the absence of forced convection, caterpillars of *E. lanestris* are able to store metabolic warmth by tightly aggregating in the tent (Ruf & Fiedler 2000). Temperature in the tent is thus influenced by the caterpillars' presence or absence. We were, however, not able to measure this effect under field conditions as these effects are overridden by environmental fluctuations of radiation and temperature. Yet, the observation that temperature

gains in tents with caterpillars were much larger (up to 25K) than in an empty tent (<5K) also emphasize the significance of the caterpillars' composite body mass for thermoregulation.

Dark bodied caterpillars may be effective behavioral thermoregulators (e.g. Porter 1982) even without a tent. Nevertheless, the use of a tent is an enormous advantage compared to individualistic or nomadic foraging caterpillar groups. The spinning of silk provides caterpillars with a material to alter their environment adaptively and consequently to improve their heat balance considerably.

For tent caterpillars of the related lasiocampid genus *Malacosoma* it has been hypothesized that the tent may function in the way of a greenhouse (Joos et al. 1988). The general characteristic of a greenhouse material should be to transmit radiation with relatively short wavelengths (such as sunlight), but block radiation of longer wavelengths (such as heat) (Goetzberger & Wittwer 1993). This tendency leads to a build-up of heat within the space enclosed by the material layer. Incoming radiation is usually absorbed by a mass enclosed in the 'greenhouse' and re-radiated in form of infrared energy. Multiple layer constructions further reduce heat loss.

Judging from our measurements of spectral characteristic of the tent material, the silk of *Eriogaster lanestris* does not match these specifications. Short-wave radiation only poorly penetrated the silk and transmittance was much lower than in transparent materials. Although we were not able to photometrically measure the transmittance of the silk for long-wave radiation ($\lambda >$ ca. 3μm) we expect the heat storing capacity of the material to be rather low, since temperature in the tent always rapidly decreased within an hour during dusk.

Obviously the tent rather functions like an ordinary tent than a greenhouse: The silk presumably absorbs a portion of the incoming short-wave radiation and emits long-wave radiation into the interior space of the tent as long as solar irradiation is sufficient. After sunset this effect expires at once and the tent cools down.

Nevertheless, the silk retards heat exchange of the caterpillars in the tent with the surrounding air (cf. Fitzgerald & Underwood 2000). The multiple layer structure should diminish convective heat loss, and air movement within intact tents has been reported to be negligible (Knapp & Casey 1986). In pine processionary caterpillars temperature gains in the tent (inhabited by the caterpillars) compared to ambient temperature were correlated to nest thickness, with only tightly woven nests gaining considerable heat (Breuer et al. 1989). These findings corroborate the notion that the transmittance of the silk does not determine the efficiency of the tent to build up high temperatures. Instead, direct heating of the silk by sunlight and the reduction of convective heat loss for the mass of caterpillars (further improved by the multi-layer structure) are responsible for the observed effect.

In contrast, in Mexican *Eucheira socialis* temperature differences between the tent's interior and the surroundings were lower in more densely woven tents (Fitzgerald & Underwood 2000). In contrast to other species investigated so far, *E. socialis* uses its tent in a completely different way, seeking out the coldest parts of the tent, exhibiting voluntary hypothermia. This may suggest that in *E. socialis* very dense silk layers nearly totally reflect incoming radiation. However, no spectral measurement data on the silk of *Eucheira* and *Thaumetopoea* are thus far available that would allow for a rigorous comparison with our data on *Eriogaster lanestris*.

It is also noteworthy in this context that the interior of the tent itself did not exhibit greatly elevated temperatures when empty which was also recorded by Knapp & Casey (1986). Evidently the thermoregulatory function of the tent primarily depends on the mass of caterpillars storing heat.

The weak transmittance of solar radiation probably explains why *E. lanestris* caterpillars always rest outside on the tent during the early morning when solar radiation is weak. Only a small amount of radiation will then penetrate the silk, too low to allow the caterpillars to thermoregulate effectively.

There is a strong trade-off between the advantages provided by the tent and its disadvantages. Costs of sociality are numerous and may even be reinforced by the tents. Among these are the fast spreading of diseases (Hochberg 1991), food shortage which may occur on small sized crippled blackthorns on xerothermic slopes (C. Ruf, pers. obs.), and the conspicuousness of the tents to visually guided natural enemies (Costa 1993). This is especially true for the tightly woven tents formed by central-place foraging social caterpillars which are among the largest and most conspicuous structures built by insects and which are visible, to humans at least, over tens of meters. Furthermore, caterpillars are in a risk to overheat in summer and may thus be forced to stay outside the tent and be even more conspicuous. Besides, building a tent means an enormous energetic investment as silk protein is costly to produce for a herbivore (Berenbaum et al. 1993).

Besides their thermal function tents of caterpillars serve other purposes like facilitating of mass assembly and recruitment to food (Fitzgerald & Peterson 1983), or reduction of water loss (Wellington 1974). Furthermore, the tent is thought to transmit vibrations to ensure cohesion of group members and to help to keep caterpillars behavior synchronous (Fitzgerald & Costa 1999).

However, the use of a tent opens up thermoregulatory capacities far in excess of those of solitarily thermoregulating caterpillars and therefore is an important selective factor for the evolution and the maintenance of sociality in *Eriogaster lanestris*. A comprehensive understanding of the thermal biology of this species will further need the integration of physiology, ecology and behavior.

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Chapter 6

Plasticity in foraging patterns of larval colonies of *Eriogaster lanestris*

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Abstract

Activity patterns of colonies of the central-place foraging (CPF) social caterpillar, *Eriogaster lanestris*, were electronically long-time recorded under field conditions. Caterpillars showed distinct, highly synchronized activity bouts during which they left their tent, fed on their host-plant and returned to their tent. Caterpillars stayed almost inactive during their digestion phases when they rested inside or on the tent.

The number of daily foraging bouts varied over a wide range. On warm and sunny days with high solar irradiation caterpillars left their tent up to nine times whereas there was only one foraging bout on cool and rainy days. On average, 4.8 daily foraging bouts were performed by fourth (=pre-final) instar larvae, which is higher than the number reported for another well known CPF species, the eastern tent caterpillar *Malacosoma americanum*. Overall, *E. lanestris* colonies required 31±2 foraging periods to pass their fourth instar within 7.3±1.2 days under the temperature conditions prevailing over the course of our experiment.

The length of individual foraging bouts as well as the phase of digestion proved to be strongly temperature dependent. In contrast to patterns reported for *M. americanum*, foraging activities were not bound to specific times of day and also continued during night time if temperature permitted. Changes in light phases only had a modulatory effect on the onset of the next foraging bout.

Eriogaster lanestris is the first lepidopteran species with CPF social caterpillars reported to show a plastic, but nevertheless strictly synchronized foraging pattern. Caterpillars are thus able to optimize their food intake and enhance growth during periods of thermally favorable conditions while minimizing their exposure to natural enemies.

Introduction

Social life-styles are an infrequent but widespread phenomenon among larvae of moths and butterflies. A number of factors have been identified which promote the evolution of social systems in caterpillars, such as social facilitation of feeding, improved defense, and thermoregulation (Costa & Pierce 1997 and references therein). However, in order to benefit from advantages accruing through sociality, behavioral requirements are necessary. This is especially relevant for synchronous behavior of individuals, which is crucial for example for tent building (Fitzgerald & Willer 1983), social thermoregulation (Knapp & Casey 1986, Joos et al. 1988, Ruf & Fiedler 2000), and recruitment communication (Fitzgerald & Peterson 1983, Ruf et al. 2001a).

Sociality of course has its costs. Behavioral synchronicity among hundreds of caterpillars means that they are extremely conspicuous to predators and parasitoids which hunt by sight and are considered the major selective agents influencing the foraging behavior of lepidopteran larvae (Heinrich 1993, Montllor & Bernays 1993). Although many species with gregarious caterpillars are aposematic and often well defended (Sillén-Tullberg & Leimar 1988), the majority of social species forage exclusively at night. Nocturnal activity, as a consequence, means doing without foraging during the thermally most suitable time of the day. Thus, for communally foraging caterpillars an extreme trade-off is to be expected between costs of synchronous mass appearance during foraging bouts and benefits of that unusual life-style. The costs should be highest in central-place foraging (CPF) caterpillars (Fitzgerald & Peterson 1988)

that must travel considerable distances between their only silk tent and their feeding places scattered in the vegetation.

Synchronicity of feeding requires cues for triggering the onset of a new activity bout. Some nocturnally feeding species simply use the onset of dusk (e.g. Weseloh 1989, Roden et al. 1990). In contrast, diurnal species may need a fixed foraging schedule independent of the thermal environment as was demonstrated in the Nearctic eastern tent caterpillar, *Malacosoma americanum*. It was hypothesized that if foraging of individual caterpillars were mediated by temperature, it would be difficult to keep activities of the colony synchronous (Casey et al. 1988). Thus, in such species the need to trigger synchronous feeding may conflict with flexible responses to suitable thermal conditions.

Previous laboratory studies on the small eggar moth, *Eriogaster lanestris*, have shown that caterpillars of this species show a sequence of strongly synchronized foraging bouts and intermediate resting phases. Caterpillars fed by day and night, and the number of foraging bouts varied with rearing temperature (Ruf et al. 2001b). These observations suggested that *E. lanestris* colonies, in contrast to all other social caterpillars studied so far, have a much more plastic foraging schedule related to environmental cues such as ambient temperature. However, laboratory studies are not necessarily transferable to field conditions. Fitzgerald et al. (1988) found that caterpillars of *M. americanum* showed an extra foraging bout at night under laboratory conditions which was non-existent under field conditions. We therefore investigated foraging activity of *E. lanestris* colonies exposed to outdoor weather conditions which allowed us to study effects of temperature over a much larger temperature range than in climate chambers.

Published observations of colony foraging patterns under field conditions in *E. lanestris* are quite inconsistent. Ebert (1994) stated that caterpillars are active by day and night or only by night, respectively. In contrast, Carlberg (1980) argued that caterpillars in Finland forage during the day only, whereas caterpillars in Germany were said only to feed at night. Instead of relying on

intermittent visual observation we here provide data from continuous long-term monitoring of the foraging patterns of colonies under field conditions. We explicitly address two questions: (a) which environmental variables determine the number of foraging bouts per day, and (b) how do foraging bouts and digestive periods change with temperature.

Material and Methods

E. lanestris is a monovoltine moth widely distributed in the Palaearctic region. Larvae hatch from egg masses in late April to early May, build a silk tent and live socially until their mid final instar. Pupation occurs in July, moths usually emerge in early spring of the next year. Host plants are various shrubs and smaller trees (*Prunus* sp., *Crataegus* sp., *Betula* sp., *Tilia* sp.). *E. lanestris* is a typical central-place forager with an elaborated communication system that facilitates the location of rewarding feeding sites and social cohesion (Ruf et al. 2001a).

Whole colonies with caterpillars of *Eriogaster lanestris* were collected in the field from a northern Bavarian population and fed with leaves of blackthorn (*Prunus spinosa*). Resulting cocoons were overwintered in a refrigerator at 5°C with interior lighting (8:16h, L:D cycle). Moths hatched within a few hours after the cocoons were warmed up in spring and were put in a plastic box (10l volume) for mating. Fresh blackthorn twigs were provided for egg deposition. Resulting egg clusters were stored outdoors in a cage sheltered from rain until caterpillars hatched. Whole egg masses with newly hatched caterpillars were placed onto small bunches of blackthorn twigs until small tents had been established. Tents were cut out of the bunches and transferred to wooden frames (cf. Fitzgerald 1980). Colonies were kept in an environmental cabinet (14:10h, L:D cycle, 20°C constant) at least until the end of the second instar. Only colonies with approximately 200 caterpillars were used for our experiments.

Colonies were then transferred to a capacious coarse meshed cage ($2.5 \times 2.5 \times$ 1.5m, width of meshes ca. 2cm) which kept birds away from the tents but did

not influence radiation, rain and wind. The cage was placed on the roof of the university building.

For quantifying activity patterns, the method presented elsewhere (Ruf et al. 2001b) to electronically record caterpillar movements between their tent and feeding sites was used. Using infrared (IR) light barriers and a specially written software program this method allows one to record events (i.e. the number of caterpillars passing the light barrier) on a computer in one minute intervals. The recording technique was modified for the use under field conditions. To prevent sunlight from influencing the infrared light barriers, we used IR detectors with integrated modulation system (type IS471, SHARP) which were connected to IR diodes (type LD274). Thus, external non-modulated radiation was not sensed by the detector. Nevertheless it proved to be necessary to put a small black plastic tube in front of the detector and shade the area of the light barrier by a narrow plastic canopy. To bundle and intensify the light emitted by the IR diode we used a plastic lens (Conrad Electronic) and a perforated dressing (shortened film tube with 1.5mm opening).

Emitter and detector were attached to adjustable aluminum sections allowing for the alignment of the light beam exactly over the plastic bridge leading from the tent to a bunch of blackthorn twigs. For each colony, two light barriers were used for double safeguarding (Fig. 1). In addition, we regularly observed foraging activities to ensure the correct functionality of the light barriers.

Twigs were replaced as needed to provide a constant supply of fresh food. The replacement of the food plant means a difference to real field conditions where food becomes more and more patchily distributed, thus forcing the caterpillars to walk over longer distances during the course of their development.

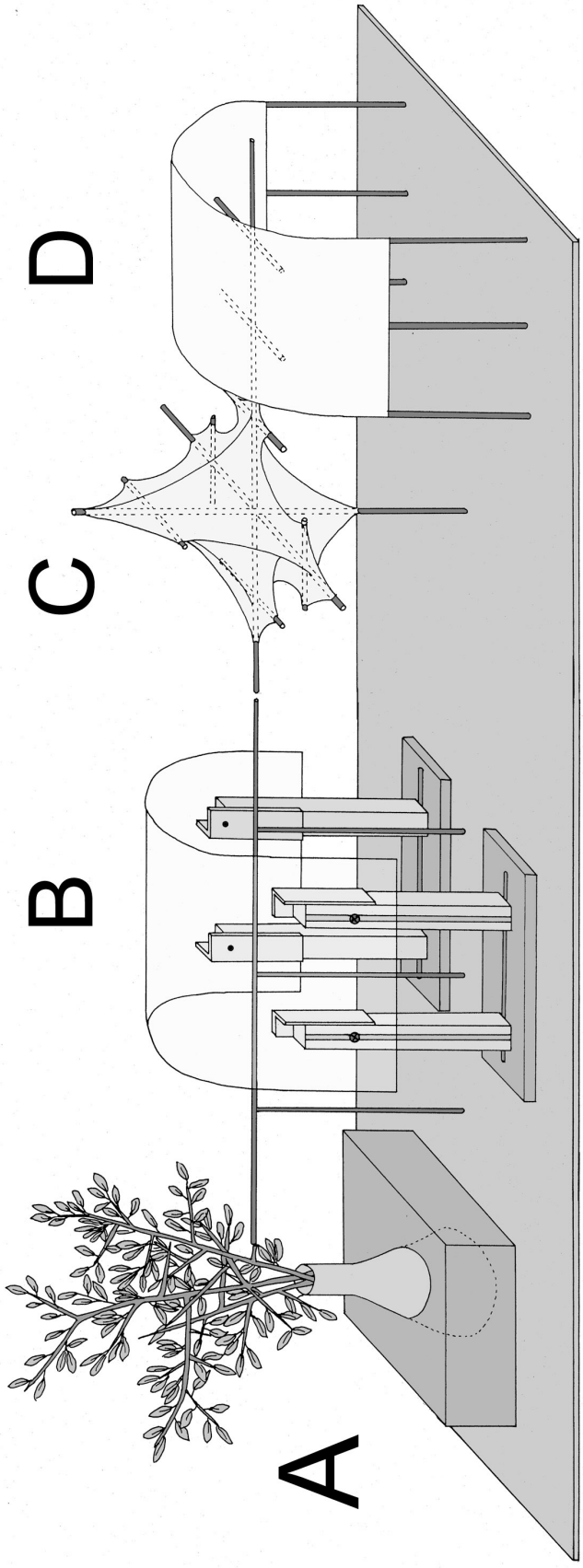

Fig. 1 – Experimental design for the registration of activity patterns of *Eriogaster lanestris* under field conditions. The whole construction is made of PVC and aluminum. **A** Bunch of blackthorn twigs in fixed vase. **B** Light barriers. L-shaped carriers of electronic components vertically, pillars horizontally movable for exact adjustment of the light barriers. Transparent roof as shelter from rain. Wires not shown for better clarity. **C** Tent built by the caterpillars on a wooden cross. **D** Cloth roof for shade on days with high radiation.

Temperature was measured to the nearest 0.1°C using high precision thermal sensors (Hygrotec SEMI 833ET calibrated thermistors) in 1min intervals. Thermal sensors were attached at a shaded position near the tents. Altogether seven colonies were monitored between 22 May and 26 June during parts of the third, the whole fourth and the beginning of the fifth larval instar.

Data for global radiation, precipitation, wind speed and atmospheric pressure (recorded at 10min intervals) were provided by a meteorological station in the Botanical Garden of the University of Bayreuth at about 300m linear distance from the study site.

All subsequent analyses are based on the foraging bouts during the caterpillars' fourth instar with data from the molting phases being excluded. During molting phases, activity of *E. lanestris* is much restrained and behavioral synchronicity is partly reduced (Ruf et al. 2001b). Activity bouts showed a bimodal form, recording the caterpillars going out to feed (first peak) and then coming back (second peak). The distinct beginning and end allowed us to accurately determine the duration of each bout. In those cases when patterns were more fuzzy we discarded single outlying signals. Out of 192 foraging periods only one foraging bout was completely disregarded because of ambiguous separation. The duration of resting phases between activity periods was assessed by determining the period between the middle of the preceding and the middle of the subsequent activity bout. This phase includes the whole interval where caterpillars rest in the tent for digestion from the point when caterpillars come back to the tent after foraging until all caterpillars have left the tent during the next foraging bout.

All statistical analyses were conducted using the STATISTICA software package (StatSoft 1999) with the exception of regression analyses that were calculated with Datafit (Ver. 7.1.44, Oakdale Engineering). Factor extraction in principal component analysis (PCA) followed Kaiser's criterion (i.e. factors were only considered if their eigenvalues were >1.0; cf. StatSoft 1999). Multiple regression analysis was performed using the standard (i.e. not stepwise) method.

Collecting multiple data at the same point may create a problem of pseudoreplication (Hurlbert 1984). However, effects of internal time series dependencies on the data were not to expected as the changing weather conditions to which the colonies were subjected did not follow a regular pattern. Moreover, colonies experienced different weather conditions during different stages of their lives since experiments were not run exactly in parallel. Finally, we were more interested in characteristics of individual foraging bouts rather than periodical phenomena, which would call for time-series analyses. Hence, foraging periods were treated as data points, and were pooled across colonies if no colony-specific differences could be noted.

Regression analyses were first calculated separately for the seven colonies. Since parameter estimates never differed significantly (95% confidence criterion), data were pooled and regression analyses were recalculated.

In many species of social caterpillars, changes of the light phases seem to be the predominant trigger for (nocturnal) activity (e.g. Schmidt et al. 1990, Floater 1996). Therefore, we also tested whether the foraging bouts in the morning or in the evening, respectively, coincide with dusk or dawn.

For all analyses, unless stated differently, temperature was averaged over the relevant time period, e.g. daily arithmetic means for analyses addressing a whole day, or means over the exact duration of the respective foraging bout for analyses on that level. Throughout the text means are given ±1SD.

Results

During the course of a day strongly synchronized activity bouts could always be differentiated from intermediate resting phases (Fig. 2, Fig. 3). This applied not only to the fourth larval instar but was also true for earlier instars and far into the fifth (=final) larval stadium (data not shown). Direct observations confirmed that each activity bout resulted in foraging. Between foraging periods caterpillars rested almost motionless in or on the tent for digestion. The number of daily foraging bouts varied enormously, ranging between one and seven during the

fourth stadium and even nine foraging periods in the third stadium (mean, fourth instar: 4.8±1.9, Fig. 3).

Even though the duration of the fourth stadium was different between colonies (min=5 days, max=9 days, mean: 7.3±1.3 days, N=7) the total number of foraging bouts required to pass this stadium was less variable (min=29, max=35, mean: 30.6±2.1, N=7).

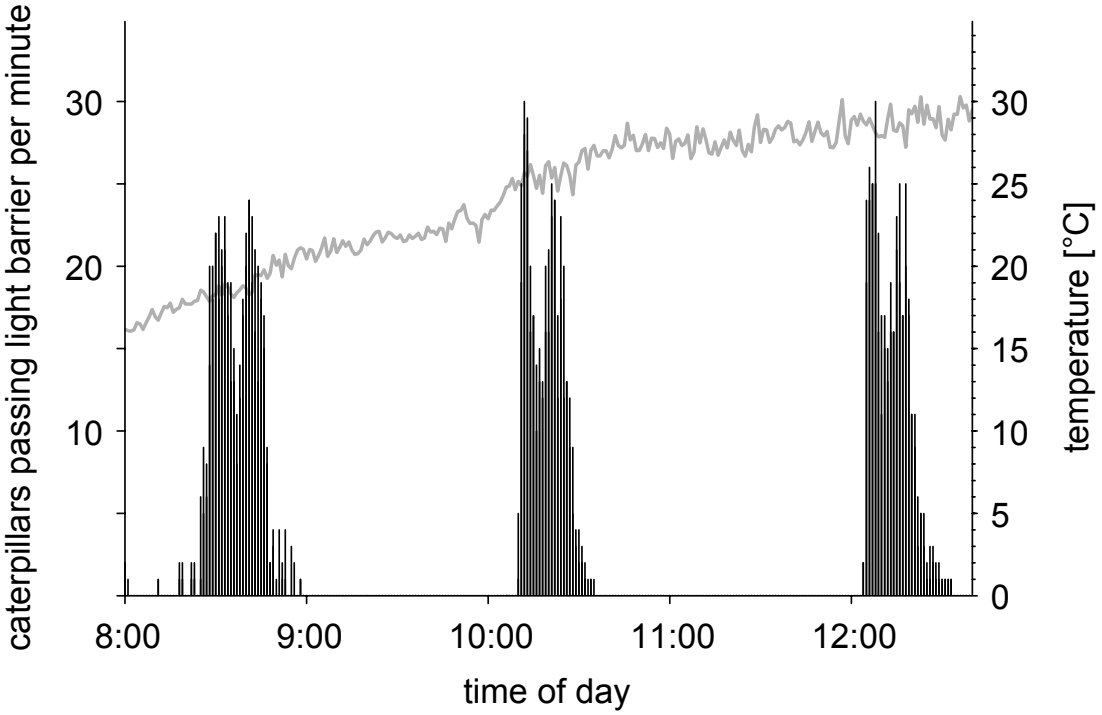

Fig. 2 – Three exemplary foraging bouts of *E. lanestris*. Distribution of signals is bimodal because caterpillars pass the light barriers twice on each foraging bout (while walking back and forth between the tent and the plant). Shown here are three foraging bouts of colony 1 (first day) at higher temporal resolution that are also depicted in Fig. 3.

We performed a PCA with daily means of temperature, radiation, wind speed and atmospheric pressure, as well as daily sums of precipitation to avoid collinearity of these climatic variables. PCA revealed that the five environmental parameters could be reduced to only two factors with factor 1 extracting 47.5%, and factor 2 extracting 21.6% of the variance. Ambient temperature, radiation and precipitation correlated with factor 1, whereas wind speed and atmospheric pressure correlated with factor 2 (Table 1).

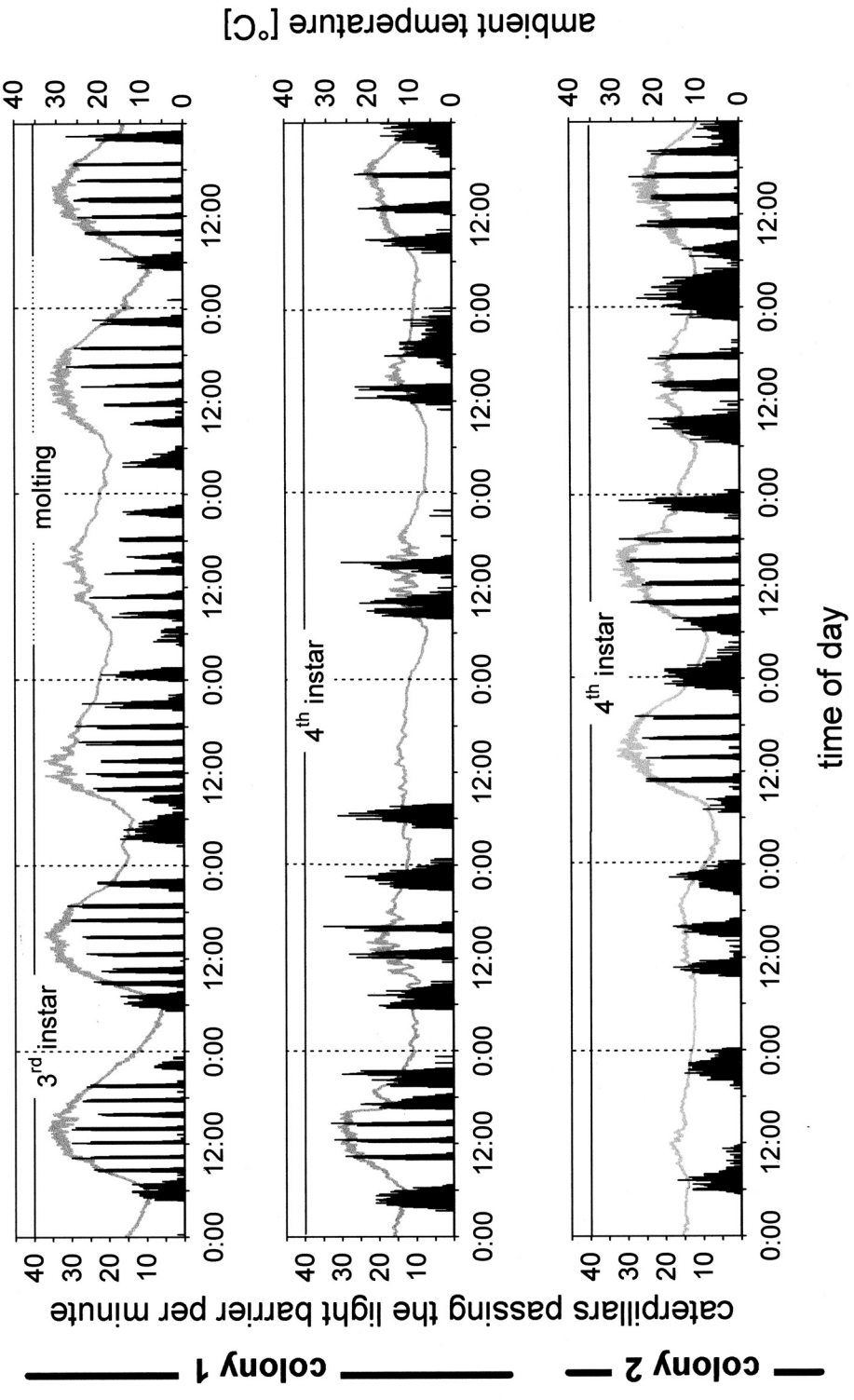
ambient temperature [°C]
3rd instar
molting
4th instar
4th instar
caterpillars passing the light barrier per minute
time of day
colony 1
colony 2
0:00
12:00
0:00
12:00
0:00
12:00

Table 1. Factor loadings of environmental variables on factors extracted by principal component analysis after varimax rotation. Marked loading > 0.7.

Variable	Factor 1	Factor 2
ambient temperature	**0.83**	-0.03
radiation	**0.85**	0.11
precipitation	**-0.77**	-0.33
wind speed	0.05	**-0.84**
atmospheric pressure	0.33	**0.72**
proportion of total variance	0.42	0.27

Multiple regression analysis with the factor values of single days extracted by PCA showed that the number of daily foraging bouts is highly correlated with factor 1 (standardized multiple regression coefficient β=0.94, t(56df)=21.7, p<0.0001) but is not correlated with factor 2 (β=0.09, t(56df)=2.0, p>0.05) (Fig. 4).

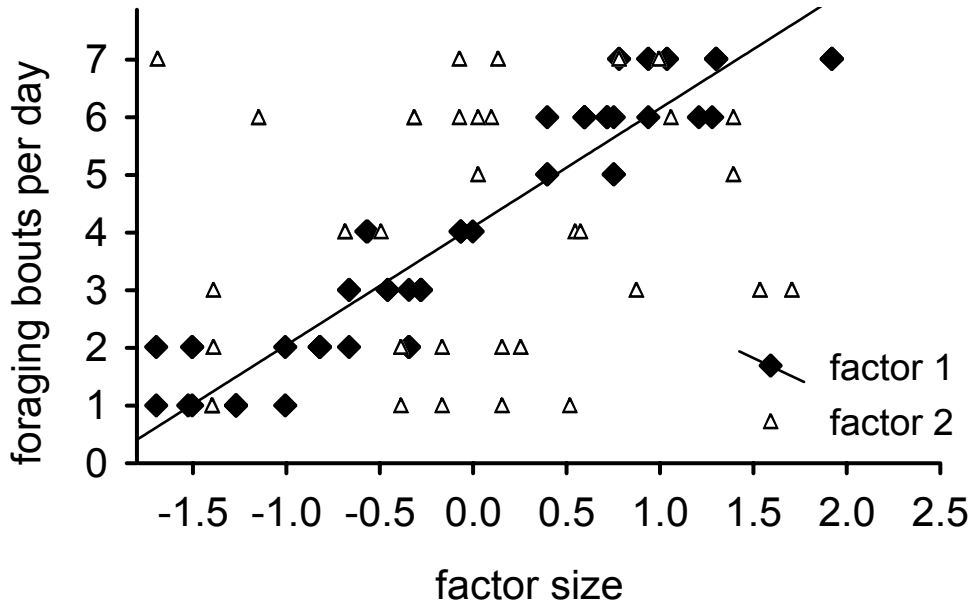

Fig. 4 – Correlation of factors extracted by PCA with the number of daily foraging bouts. Factor 1 includes ambient temperature, radiation and daily precipitation, factor 2 includes wind speed and atmospheric pressure. Linear regression for factor 1: y=4.10+2.05x.

Thus foraging patterns strongly depend on weather conditions. On sunny days caterpillars of a colony may forage several times a day (upper panel in Fig. 3) and therefore enhance nutrient uptake whereas foraging is strongly restricted during periods of low radiation associated with low temperatures and rainfall (e.g. middle panel in Fig. 3).

Temperature affected foraging in two ways: First, the duration of each foraging bout was strongly influenced by temperature. Foraging bouts lasted less than one hour on average at 25-30°C (Table 2), but lasted two to five hours when temperatures fell below 15°C (Fig. 5A; regression for pooled data: R^2=0.76). This effect was mainly caused by the fact that temperature directly reduces velocity of the caterpillars crawling between their tent and their host plant and slows down food uptake. Although the duration of foraging bouts seemed to decrease steadily with increasing temperature, categorization of temperature revealed that there was no significant difference between foraging bouts occurring at either 20-25°C or 25-30°C (Table 2) indicating that physiological thresholds limit the maximum speed of foraging attainable.

Table 2. Durations of foraging bouts and digestion phases categorized by temperature. All values are means±SD. Different letters in superscript indicate significant differences between categories within one column after Tukey-Kramer test following a Kruskal-Wallis test to assess column-wide significance.

Temperature range	Duration of foraging bout [min]	Duration of digestion interval [min]
< 10°C	189.2 ± 69.0^a (N=9)	550.0 ± 171.3^a (N=8)
10°C ≤ x < 15°C	176.9 ± 58.0^a (N=46)	466.7 ± 186.9^a (N=46)
15°C ≤ x < 20°C	90.5 ± 30.6^b (N=37)	265.6 ± 99.0^b (N=38)
20°C ≤ x < 25°C	52.1 ± 21.0^c (N=47)	201.2 ± 56.0^c (N=47)
≥ 25°C	42.8 ± 17.1^c (N=52)	144.4 ± 17.2^c (N=49)
Test statistics	H(4df)=140.9, p<0.0001	H(4df)=143.5, p<0.0001

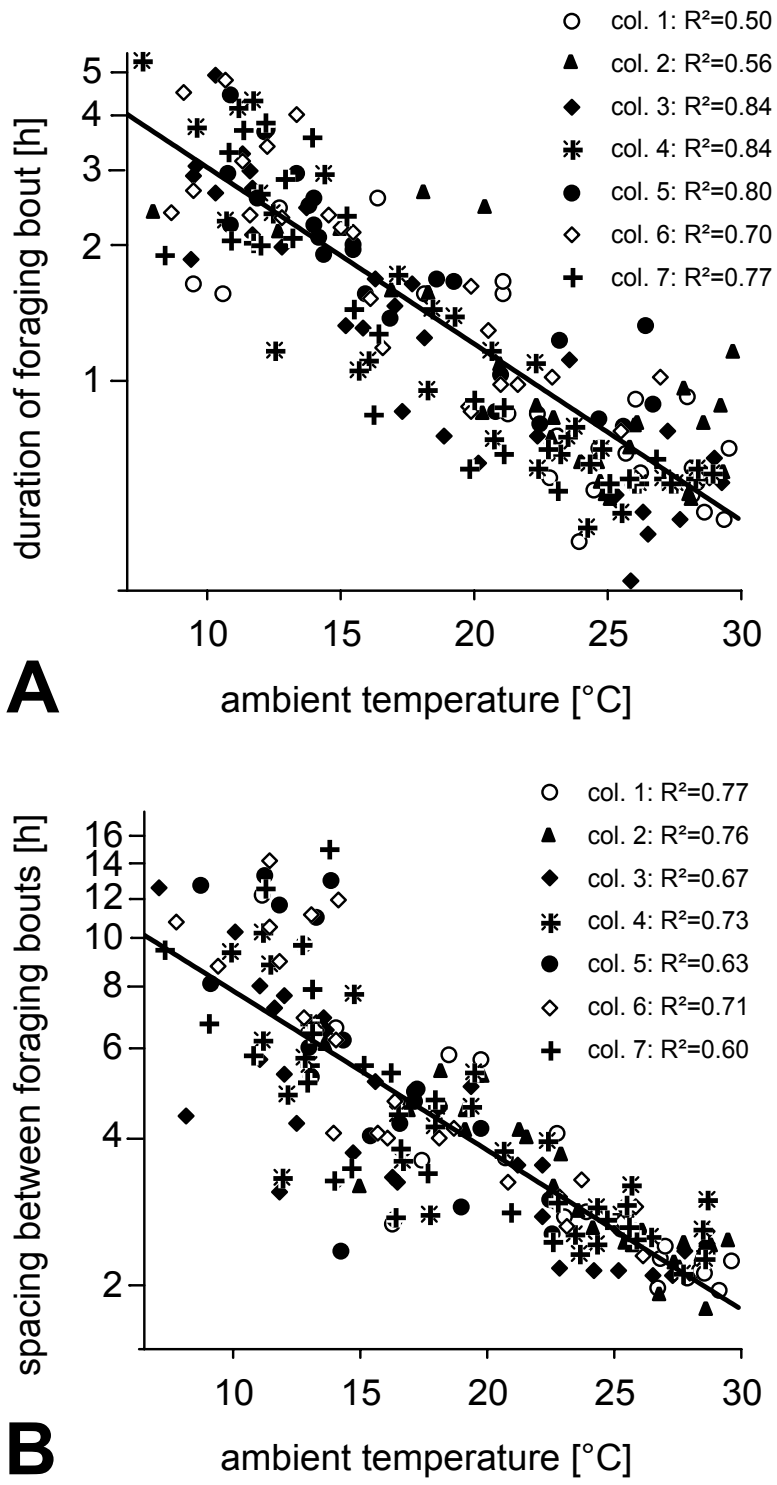

Fig. 5 – Duration of foraging bouts.(**A**) and spacing between foraging bouts (**B**) at different ambient temperatures. In order to linearize the relationship and homogenize variances data were log transformed for statistical analyses. Parameter estimates did not differ significantly between colonies (col.). Regressions were statistically significant for individual colonies ($p<0.01$). Regression for pooled data (bold line): (A) $y=-0.040x+2.66$, N=191 (B) $y=-0.032x+2.99$, N=188.

Temperature had an even more intense effect on the period between the foraging bouts which is the phase for digestion, when caterpillars rest in or on the tent. This period varied between 2-4h at 20-30°C, but increased to far more than 10h and became unpredictable at lower temperatures (Table 2, Fig. 5B; regression for pooled data: R^2=0.71).

Although activity patterns seemed to be largely controlled by thermal factors, the light regime might also modify this pattern. Figure 6 shows that the distribution of the starting times of activity bouts was irregular over the course of a day.

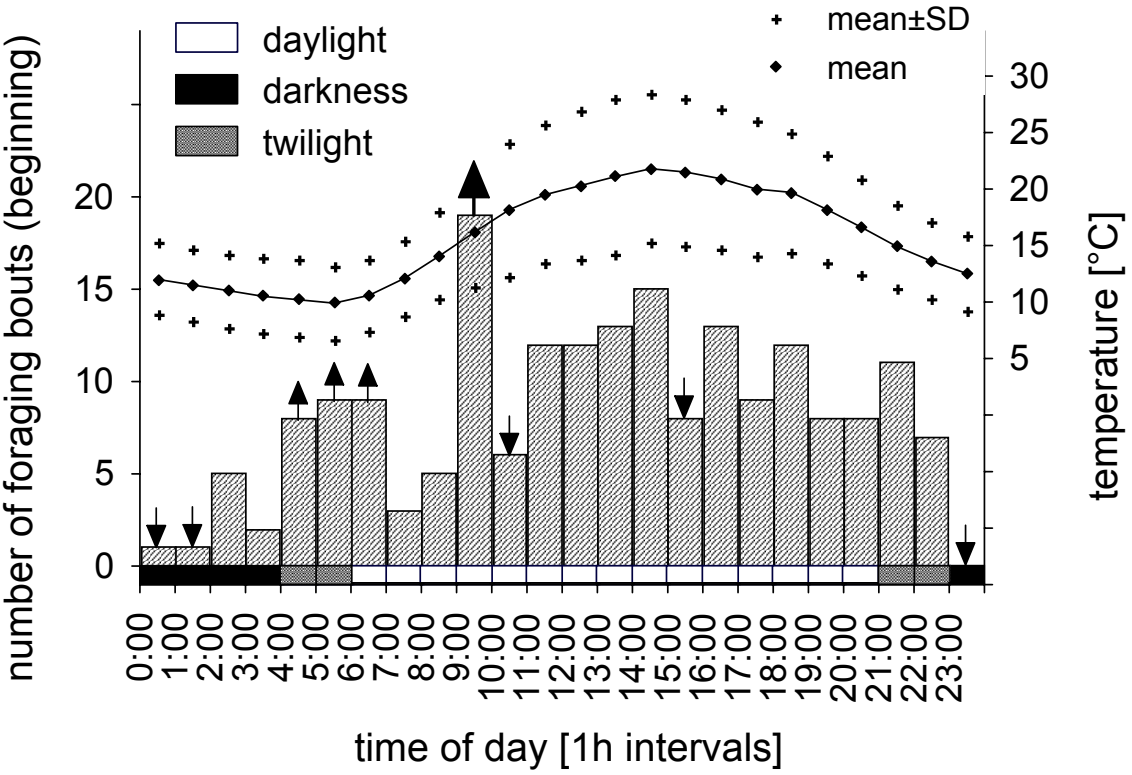

Fig. 6 – Incidence (starting point, CEST) of 196 activity bouts pooled for seven colonies of *E. lanestris* relative to the time of day. Means (black diamonds and line) and standard deviations (crosses) of daily air temperatures recorded in the shade at the study site during the study period. Arrows mark hours of day which show more or fewer events than would expected after linear regression of number of foraging bouts on mean temperature (R=0.62, p<0.01, y=–2.81+0.70x). Small arrows ≥1 standardized residual value (srv), large arrow ≥2 srv.

Ambient temperatures averaged for 1h intervals over all measurements taken over the entire experimental period were significantly correlated with the number of foraging bouts that started during these same 1h intervals (R^2=0.38, p<0.01), but a disproportionately large number of foraging bouts occurred just after sunrise and again between 0900 and 1000h. In contrast, there was a disproportionately low number of foraging bouts during the first hours after sunset as well as during the interval right after the 0900h peak and between 1500 and 1600h. Nonetheless, there was no well-defined accumulation of foraging bouts at any time of the day that would occur every day. In particular, nocturnal foraging periods did occur under outdoor conditions in nights with favorable temperatures (nightly mean: 13.6°C, min=6.6°C).

Obviously an intrinsic fixed activity pattern controlled by light, if present at all in *E. lanestris*, is strongly superimposed by environmental conditions. On cold and rainy days, when temperatures hardly rose above 10°C, caterpillars left the tent at least once every day. Under such conditions, without the strong acceleration of foraging activity due to solar radiation, one might expect light-phase triggered activity patterns to be more pronounced. Figure 7 shows that the first foraging bout on these days did not coincide with sunrise either, further corroborating the observation that foraging is mainly influenced by thermal conditions.

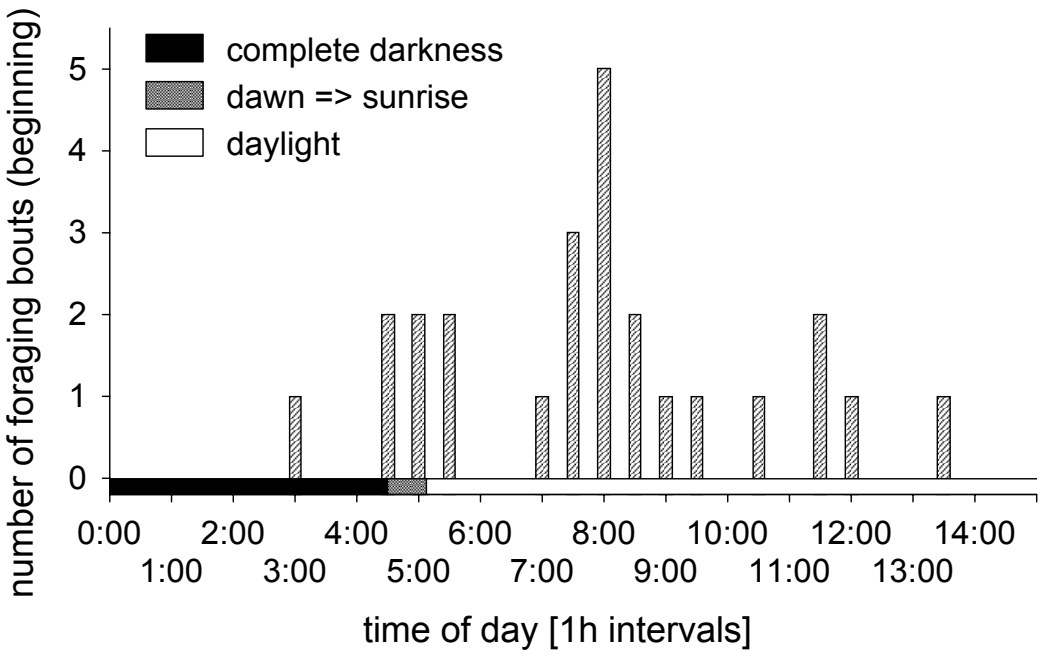

Fig. 7 – Starting time (CEST) of the first foraging bout of a day. Only cold and rainy days with three or fewer daily foraging bouts were taken into account. Times for dawn and sunrise are averaged and varied for less than 10min over the study period.

Discussion

The general foraging pattern of *E. lanestris* caterpillar colonies under field conditions was quite similar to that reported for laboratory colonies (Ruf et al. 2001b). Despite large temperature variation and occasional rain fall, activity periods proved to be perspicuously synchronized and distinct digestion phases were always well separable from foraging activities outside the tent. Caterpillars of *E. lanestris* were active by day and night, but never fed during the night time if temperatures were too low (well below 10°C). This may explain the totally diurnal behavior of Finnish populations (Carlberg 1980). We did not observe concentrations of foraging events at special times of day, which is in accordance with Carlberg (1980) and Balfour-Browne (1933) who both stated (without presenting supportive data) that the larvae showed no special foraging and resting times.

In contrast to all other social tent building caterpillars investigated so far showing CPF strategy (Table 3), the number of foraging bouts per day was neither constant nor restricted to special times of day but proved to depend on the weather. Solar radiation and temperature had the most pronounced effects, while precipitation, wind and light regime were of modulatory significance at most. The total number of foraging bouts achievable during one day depends on both the rate at which caterpillars forage and the rate of digestion.

The duration of foraging bouts increased steadily with decreasing temperature. The reasons for this sharp increase are probably numerous. First, locomotion and feeding efficiency of caterpillars are severely constrained at low temperatures (cf. Joos 1992).

In addition, caterpillars do not forage independently of each other but use a trail system (cf. Ruf et al. 2001a). Caterpillars' advancement during trail following is probably severely restricted at low temperatures, because they are expected to need more time at each ramification to decide for one of the trails, and these effects will sum up.

As bunches of the host plants were replaced regularly over the duration of the experiments, we destroyed existing trails and caterpillars afterwards strongly hesitated to crawl on the new branches. This accounts for part of the variability at any given temperature and might be even more pronounced at low temperatures due to slow marking of a new trail.

In ectothermic animals, temperature has an overwhelming effect on all physiological processes including digestion (Schroeder & Lawson 1992, Casey 1993 and references therein). The duration of the resting phase between the foraging bouts which is largely equivalent to the digestion phase proved to be strongly temperature dependent in our experiments. We did not verify whether caterpillars of *E. lanestris* had completely digested the food they had consumed during the previous foraging bout when leaving the tent again. However, the temperature dependence of duration of periods spent in or on the tent between two foraging bouts strongly suggests this, because one would not expect such

close temperature dependence with phases of resting behavior only. Complete digestion of a full gut's load of food within a minimum of 2-3h at ambient temperatures of 25-30°C is absolutely within the realms of possibility (cf. Fitzgerald et al. 1988) since caterpillars of *E. lanestris* regulate their body temperature between 30-35°C under sunny weather conditions (Ruf & Fiedler 2002). Thus, caterpillars seem to benefit from favorable weather conditions by maximizing their rate of food intake up to a physiological threshold, when no further acceleration of digestion is possible. Neither the duration of foraging bouts nor of digestion periods showed a substantial increase at ambient temperature above 20°C, suggesting that due to their combination of solar basking and tent-based thermoregulation caterpillars in the temperature range of 20-30°C approach their physiological optimum.

In contrast to *E. lanestris* larvae, Eastern tent caterpillars, *Malacosoma americanum*, show a fixed activity pattern with foraging bouts occurring at around 0600h, 1500h, and 2000h (Fitzgerald et al. 1988). Thus, caterpillars begin foraging bouts at a definite time of day even when their gut might still be partially filled with undigested food from the previous foray. Indeed, when caterpillars were held for six hours at 15°C they showed no indication of having processed food (Casey et al. 1988). On the other hand, these caterpillars are also unable to benefit from a high temperature by means of abridged pauses between forays and stays in the tent even though their gut may be completely empty. Therefore, the foraging strategy of *E. lanestris* seems to be more advanced with regard to opportunistically taking advantage of favorable temperature conditions.

Anyway, individual caterpillars of *E. lanestris* are expected to exhibit a variable range of digestive conditions at the beginning of each foraging bout. This is due to the fact that the re-arrival of some caterpillars at the end of the previous foraging bout is often quite delayed and caterpillars face different body temperatures at different locations in the tent (cf. Joos et al. 1988). Thus, individual caterpillars might also be physiologically "out of phase" when they start a new foraging bout.

Tent building caterpillars are well known for their thermoregulatory abilities. It has been shown repeatedly that especially social caterpillars are able to elevate their body temperatures significantly by basking and that temperature gains are much higher than those of solitary caterpillars (Knapp & Casey 1986, Joos et al. 1988). Raising the body temperature by basking speeds up physiological processes enabling larvae to consume and digest food faster and, thus, develop more quickly. It is obvious that thermoregulation is most advantageous if caterpillars really benefit from their accelerated digestion by foraging more often than would be possible if a caterpillar's body temperature were to track the surrounding air temperature during digestion. Assuming that caterpillars of *E. lanestris* need an average of 31 foraging bouts to complete their fourth larval stage, this species would need 10 days if they had a fixed foraging schedule with only three foraging periods per day provided that they consume the same amount of food during each foraging bout. Due to their plastic foraging patterns caterpillars are able to half this time when temperature conditions are favorable. Consequently, *E. lanestris* is well adapted to undergo rapid larval development under unpredictable spring weather conditions in Central Europe when temperature is often low during daytime but radiation is frequently intense (e.g. on cool but sunny mornings).

Of course, foraging patterns of insect herbivores are not exclusively affected by the prevailing thermal conditions. They are also influenced by the availability and abundance of suitable food (Slansky 1993) and by the presence of natural enemies (Stamp & Bowers 1988). In our experiments fresh food was always available in ample supply in close proximity to the tent and natural enemies were largely excluded. Nevertheless, the latter does not influence innate behavioral patterns, i.e. if predator avoidance is obligatory and does not depend on experiencing contacts with natural enemies.

Casey et al. (1988) suggested that the fixed foraging schedule of *Malacosoma americanum* was necessary to synchronize individuals. In contrast, *E. lanestris* caterpillars are able to synchronize their foraging activities despite variable foraging times which supports the assumption of Fitzgerald et al. (1988) that the

foraging pattern of *M. americanum* is primarily shaped by predator avoidance. This latter hypothesis is further supported by the observation that last instar caterpillars of *M. americanum* become strictly nocturnal and forage independently of their tentmates. Predator avoidance is generally thought to be the cause of completely nocturnal activity in caterpillars (Heinrich 1993). Many caterpillars remain hidden during the day and many species with social tent building CPF caterpillars are strictly nocturnal with the exception of the genera *Malacosoma* and *Eriogaster* (Table 3). Hence, all such nocturnal caterpillars defer their foraging activities until periods with lower predation risk, but also characterized by lower temperatures which are less favorable for foraging and digestion.

There is a trade-off between the risk of attack by visually oriented predators or parasitoids and the necessity to grow fast which is especially strong in early spring caterpillars that rely on young leaves as food source (cf. Stamp & Bowers 1990, Parry et al. 1998). It is to the caterpillars advantage to complete their development while their food supply is still of high quality (cf. Slansky 1993). A plastic foraging response is generally interpreted as enhancing growth rates by increasing rate of feeding and digestion at higher body temperatures. Of course, frequent foraging during the day raises the caterpillars' conspicuousness and large groups of larvae are certainly easier for enemies to find than solitary caterpillars. However, foraging periods are especially short during the day (i.e. at high temperatures). This spatio-temporal coordination may limit colony losses to predators because individual caterpillars profit from the security of the group (Hamilton 1971) and synchronicity also reduces the absolute time the caterpillars are outside the tent. Even fifth instar caterpillars of *E. lanestris* retain tent-based communal foraging, although group cohesion diminishes progressively as the caterpillars approach the prepupal stage (cf. Ruf et al. 2001b).

Table 3. Activity patterns of 13 lepidopteran species with social, tent building, central-place foraging caterpillars. Most assessments of activity patterns rely on intermittent visual observations.

Species	Familiy	Study site	Activity pattern	References
Eriogaster lanestris	Lasiocampidae	Laboratory + field	Day and night, **plastic**	Ruf et al. 2001b, this study
Eriogaster arbusculae	Lasiocampidae	Field	Diurnal	ProNatura 2000
Eriogaster catax	Lasiocampidae	Laboratory	Day and night	Ruf, unpubl. obs
Eriogaster philippsi	Lasiocampidae	Field	Diurnal	Talhouk 1940
Eriogaster amygdali	Lasiocampidae	Field	Diurnal	Talhouk 1975
Malacosoma americanum	Lasiocampidae	Laboratory + field	Day and night, **fixed schedule**	Fitzgerald 1980, Fitzgerald et al. 1988
Malacosoma neustria	Lasiocampidae	Laboratory	Day and night	Ruf, unpubl. obs.
Gloveria sp.	Lasiocampidae	Laboratory	Nocturnal	Fitzgerald & Underwood 1998a
Eutachyptera psidii	Lasiocampidae	Laboratory + field	Nocturnal	Sallé 1856, Comstock 1957
Ochrogaster lunifer	Thaumetopoeidae	Field	Nocturnal	Floater 1996
Thaumetopoea pityocampa	Thaumetopoeidae	Field	Nocturnal	Schmidt et al. 1990
Thaumetopoea processionea	Thaumetopoeidae	Field	Nocturnal	ProNatura 2000
Eucheira socialis	Pieridae	Laboratory + field	Nocturnal	Kevan & Bye 1991, Fitzgerald & Underwood 1998b, Fitzgerald & Underwood 2000

Unfortunately, there are no reliable data on the impact of natural enemies on the survival of *Eriogaster lanestris* in the field. In our samples, few (<10) of several hundred individuals collected in the field were parasitized by tachinid flies. Vespid wasps (*Polistes* sp.) and ants (*Formica* sp., *Lasius* sp.) were observed to attack caterpillars only when they were forced to stay outside the tent at high ambient temperatures. Last instar *E. lanestris* caterpillars that are probably at most risk of succumbing to vertebrate predation possess reddish, urticarial hairs which cause severe contact dermatitis, at least in humans (Kawamoto & Kumada 1984). The impact of these hairs on birds and small mammals has never been investigated, but judging from their strong effect on humans, mature *E. lanestris* larvae are probably quite well defended. Improved chemical defense, accompanied by a putatively aposematic black and reddish coloration of final instar larvae, might well explain why *E. lanestris* seems to have been able to adopt a more flexible, but also more risky, foraging strategy in comparison to *M. americanum*.

Extrinsic factors other than climatic parameters (e.g. light phases) had only a subsidiary effect on the onset of a new foraging bout with a slight accumulation of foraging bouts during the first daylight hours but no peak of activity right after sunset. This occasional feeding during the coldest hours of a day might point to an early filling of the gut to benefit from good digestive conditions in the subsequent morning hours. Despite the fact that the state of digestion probably differs among individuals at the onset of each foraging bout, how caterpillars of *E. lanestris* manage to coordinate their activities without relying on extrinsic timers is not yet known.

Overall, the plastic foraging pattern of *Eriogaster lanestris* shows a striking example for the interaction between physiology, behavior, and ecology. Comparing *E. lanestris* and *M. americanum* shows that despite many very similar life-history traits (such as voltinism, tent building behavior, and early spring feeding) different behavioral strategies may arise. Much of our current understanding of sociality in lepidopteran caterpillars stems from studies of tent caterpillars in the genus *Malacosoma* (Fitzgerald 1995). However, comparative

investigations on different systems under a range of ecological conditions are necessary to critically test the generality of patterns and processes that govern the evolutionary ecology of social foraging in caterpillars.

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Chapter 7

Trail-based Communication in Social Caterpillars of *Eriogaster lanestris*

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Abstract

Caterpillars of *Eriogaster lanestris* (Lepidoptera: Lasiocampidae) mark trails as they move between feeding sites and their communal tent. They prefer new trails over aged ones. Hungry caterpillars prefer trails marked by fed caterpillars returning to the tent. Thus successful foragers direct tentmates to profitable food finds, in a manner similar to *Malacosoma americanum*. *E. lanestris* readily follows trails prepared from 5β-cholestane-3-one, a component of the trail marker of *M. americanum*, when applied at a rate of $\geq 10^{-10}$g/cm. In choice tests, they preferred more highly concentrated over weaker trails. New trails are always established in groups and velocity increases from early to late caterpillars traversing a new trail.

Introduction

Although trail marking and trail following is usually associated with eusocial insects (e.g. ants and termites) it has been documented for a number of other taxa including gastropods, spiders, bugs and some larval Lepidoptera (Roessingh 1989 and references therein). In ants trail-based communication is often highly sophisticated and allows the highly coordinated foraging, leading to high efficiency in locating and exhausting food patches (Hölldobler & Wilson 1990). Much less work has been done on non-eusocial insects and diversity and efficiency of communication systems of these groups remains poorly understood. At present the communication systems of only about 3% of social lepidopteran species have been examined. Larval communication has been

analyzed in perhaps the greatest detail in the tent caterpillar *Malacosoma americanum* (Fitzgerald 1995). The trail system of this species contains substantial information. Successful foragers use a pheromone and direct siblings to the most profitable food finds, showing obvious parallels to food recruitment strategies among ants and termites (Fitzgerald & Peterson 1983; Fitzgerald & Costa 1999).

Fitzgerald and Peterson (1988) classified social caterpillars as patch restricted, nomadic or central-place foragers depending on whether they feed within their home webs, travel between different resource patches, or use a communal nesting site which they leave periodically to forage. These authors hypothesized that caterpillars foraging from a fixed home base are more likely to evolve sophisticated systems of communication to contend with increasingly depleted and patchy resources. However, studies on *Gloveria* sp. (Lasiocampidae) and *Eucheira socialis* (Pieridae) (Fitzgerald & Underwood 1998a,b) revealed that central-place foragers do not always exhibit fine-tuned communication systems and ecological constraints (e.g. spatial distribution of acceptable food patches on host-plants) might influence the evolution of communication.

The family Lasiocampidae itself presents a diversity of group-foraging strategies, including nomadic and central-place foragers, that differ in details of shelter-building and chemical communication (Fitzgerald 1995). Behavioral studies from a broader array of social lasiocampids are needed for a comparative study of social evolution in this group, and may yield insight into mechanisms that influence the parallel evolution and diversity of social behavior in social caterpillars in general. To this end we undertook studies of trail following behavior in larvae of the Palaearctic small eggar moth *Eriogaster lanestris*.

Materials and Methods

Insect collection and rearing: Caterpillars of *Eriogaster lanestris* (Linnaeus, 1758) eclose in early spring just after budbreak and live together in silken tents on blackthorn (*Prunus spinosa*, Rosaceae) or other deciduous trees and shrubs

(e.g. *Crataegus* spp., *Betula* spp., *Tilia* spp.) (Ebert 1993). Tents are maintained throughout larval development and are abandoned shortly before pupation (Balfour-Browne 1933). This species is a central-place forager, leaving the tent to forage several times a day.

Whole colonies of about 50 to 200 individuals were collected from blackthorn (*Prunus spinosa*) near Würzburg, Germany, and transferred to environmental chambers with a 14:10h (L:D) light regime. Temperature ranged between 15°C (night) and 22°C (day). Caterpillars were taken in their original tents into the laboratory, where each whole tent was placed in a new bunch of blackthorn twigs. Subsequently, the larvae extended their tent to include these new twigs. The caterpillars were permitted to adjust to the new surroundings for three days, after which all remaining leaves were removed from the twigs around the tent and caterpillars were given access to a bridge leading to fresh food. Bridges were made of wooden dowel rods of 5mm diameter and 50cm length. Cut sections of such bridges (5cm each) were the basis for all trail following tests. Fresh food was offered in ample supply twice every day. Experiments were conducted with penultimate (fourth) and ultimate (fifth) instar caterpillars.

Bioassays: All bioassays were conducted with Y mazes made of wood (cf. Weyh & Maschwitz 1978). To prevent the caterpillars from leaving the maze, rods were raised about 2cm above the substrate by small, rounded pieces of wood. No wood was placed beneath the point of contact of the three rods to exclude the possibility that any silk or pheromone left by a preceding caterpillar would influence the decision of a subsequent caterpillar. Each test bridge was only used for one experiment.

Caterpillars were allowed to select between two trails (marked by the caterpillars themselves or prepared with silk or synthetic pheromone, see below). Choice for one of the two trails was considered valid when a caterpillar had completely passed onto one of the two rods. The sides of the two different trails were always switched after half of the experiments to control for any side preference. The stem of the Y was reused in the experiments and the trail was

therefore well established, facilitating caterpillars' movement before reaching the choice point.

For bioassays we only used caterpillars that started walking on their tent at the beginning of a new foraging period, which is a clear indication that they are hungry and motivated for finding food (for *Malacosoma* see Fitzgerald 1995). From every colony only 10-12 caterpillars were removed for each series of bioassays to avoid influencing the course of colonial foraging. All test series involving 10-12 caterpillars each were replicated 3-10 times. Within every test series each caterpillar was only used once. Afterwards, test caterpillars were allowed to feed and re-integrate into their home colony.

Preparation of trails: In a series of preliminary experiments we tested if caterpillars of *E. lanestris* preferred trails naturally marked during one foraging period, or trails artificially prepared with silk from the tent, over totally unmarked trails. Naturally marked trails were obtained by cutting the rods leading to the host plant in small sections. Rods prepared with silk were obtained by turning fresh rods through the silk at the edges of the tents. As manually prepared trails were strongly covered with silk while trails marked by the caterpillars showed only thin strands of silk we subsequently tested which of these two trail types caterpillars preferred.

To find out whether caterpillars of *E. lanestris* are able to distinguish trails leading to profitable or unproductive feeding sites, respectively, we used the following experimental design. The bridge leading to the host plants used for the experiments consisted of a proximal stem section leading to a small platform where a distal arm was attached at an angle of about 20° (Fig. 1). At the beginning of a foraging period caterpillars were allowed to establish a trail along the arm which led to a totally defoliated branch of the host plant (unproductive feeding site). Exploration of the 50cm long trails lasted 15-30 minutes. Ten minutes after caterpillars had reached and explored the defoliated branch a second arm was joined to the platform, now forming a Y, the arms of which were separated by an angle of 40°. Caterpillars were then able to establish a new trail to a foliated branch (profitable feeding site), where they were allowed

to feed to repletion and return to their tent. When the last caterpillar had reached the tent both rods were removed and cut into pieces used for the bioassays. Differences in trail age between the two rods were considered negligible because the old trail was not immediately abandoned in favor of the new trail and the bioassays were not conducted until the spontaneous onset of the next foraging period of the same colony, which was about four to five hours later.

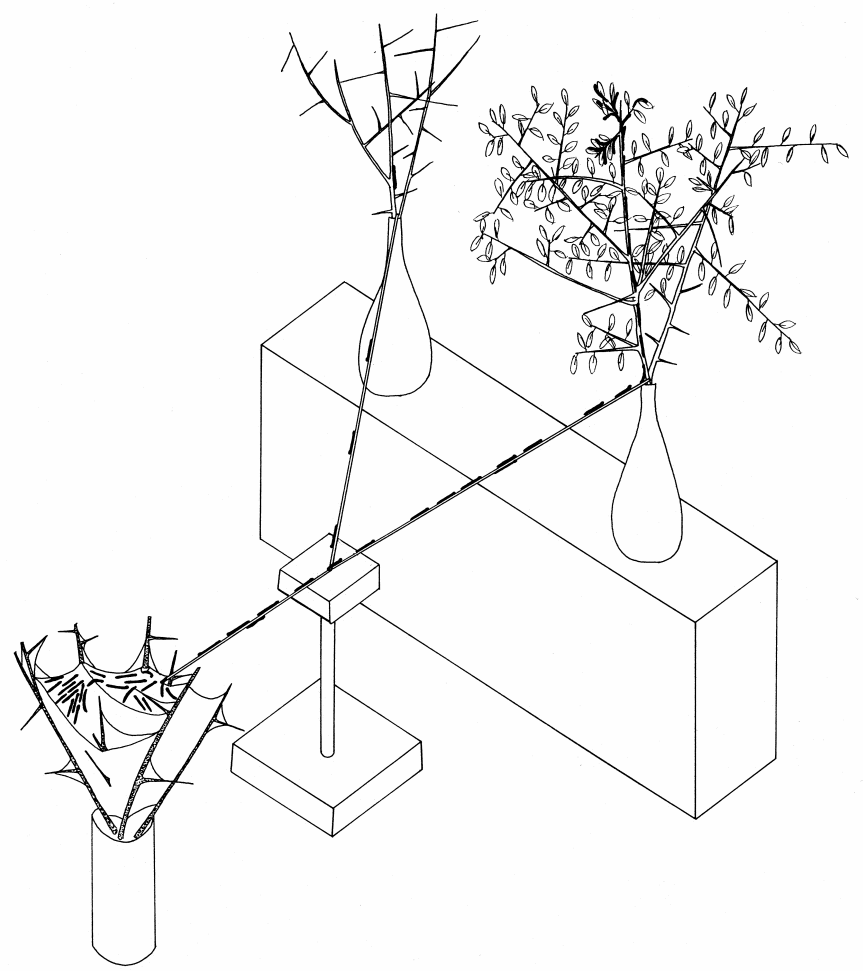

Fig. 1 – Experimental design for preparing trails leading to unproductive or profitable feeding sites respectively. Rods were made of wood (length: 50cm).

To determine if the caterpillars are able to asses trail age, caterpillars were allowed to lay a trail during one foraging bout. This trail was then set aside, cut into pieces and aged until the next day. During the course of the first foraging bout on the next day a second, new trail was obtained as described above. At

the beginning of the subsequent foraging period caterpillars had to choose between old trails (about 20 hours old) and new trails (4-5 hours old).

Site of secretion of the trail marker: To test the possibility that *E. lanestris* uses an extrasilk trail marker as was reported for other social caterpillars we modified the experimental design of Fitzgerald and Underwood (1998b). A 10 × 6cm-sheet of paper was folded along both diagonals. Parts of the creases were marked 1cm in one direction and 3cm in the other direction starting from the crossing point. To obtain trails, the venter of the tip of the abdomen of one caterpillar was dragged several times along one of the creases between the markings. The sheet was then unfolded and a caterpillar was placed on the paper with its head between the crossing of the two creases. Swinging its head from side to side the caterpillar touched both creases and could therefore find the prepared trail. A positive response was scored when the caterpillar followed the trail to the mark within three minutes after it started to search for a trail.

Pheromone tests: Weyh and Maschwitz (1978) demonstrated that trail following in *Eriogaster lanestris* is mediated by some chemical factor. We therefore tested if the trail pheromone 5β-cholestane-3-one, identified by Fitzgerald and Webster (1993) to be a component of the trail marker of tent caterpillars, elicits trail following in *E. lanestris*. We assessed the sensitivity of the caterpillars to different concentrations of the pheromone. 5β-cholestane-3-one (Sigma Chemical Company No. C 2152) was dissolved in hexane and the solution diluted from 10^{-6}g/cm to 10^{-10}g/cm trail. 50µl of the pheromone solutions of different concentrations were applied to one arm of the Y, while pure hexane was simultaneously offered at the other. We also tested the response of the caterpillars when confronted simultaneously with two concentrations of the pheromone applied to the alternate arms of the Y using 10-fold concentration differences. To assess the importance of mechanical guidelines for trail following the pheromone solution (4×10^{-10}g/cm) was laid out in an S-shape on filter paper. Single caterpillars were allowed to crawl on the paper and the position of the caterpillar's head capsule was registered in a rough drawing on a

second paper. Each caterpillar was also tested on trails prepared with pure hexane as a control.

Velocity measurements: To determine if well-explored trails facilitate movement of the caterpillars or if new, unexplored trails slow down the progression to food sources we measured velocity of as many caterpillars as possible crawling between their tent and the host plant. For this purpose we measured with a stop watch the time taken by caterpillars to walk on the rods for the longest distance observable. Distances were determined by dividing the rods into 5cm sections by markings with a ballpoint pen. Markings were used later for cutting sections for the Y-test bioassays. Caterpillars that were disturbed by their tentmates and therefore slowed down or stopped were disregarded. Caterpillars were categorized in five groups: “Scouts” start exploring a new trail but turn back before reaching the host plant. “First five out / back” are the first five caterpillars reaching or leaving the plant. “Others out / back” are all caterpillars reaching or leaving the host afterwards.

Statistics: Statistical analyses were conducted using the software package STATISTICA (StatSoft 1999). Frequency tables with several replicates of one experimental series were analyzed by adding up χ^2 values calculated individually for each experiment. Significance was then assessed with the number of test series as degrees of freedom (Sachs 1992). The number of individual trials for each series (N) is also given.

Results

In all of three replicates caterpillars clearly preferred trails prepared with silk of the tent to unmarked rods (χ^2(3df, N=36)=25.66; p<0.001). Furthermore 51 of 60 caterpillars opted for trails marked by caterpillars during one foraging period rather than unmarked ones (χ^2(6df, N=60)=30.0; p<0.001). Caterpillars preferred naturally marked silk trails over artifically prepared trails, despite the greater silk cover of the artificial trails (χ^2(6df, N=60)=42.0; p<0.001).

In most of the ten test replicates the majority of caterpillars chose the more recent of two differently aged trails (Table 1). Overall 77 out of 100 caterpillars

preferred newer trails and pooled results of the tests are highly significant (χ^2(10df, N=100)=38.8; p<0.001).

Table 1. Test series A: Number of *Eriogaster lanestris* caterpillars choosing aged vs. new trails. Test series B: number of caterpillars choosing trails marked by hungry caterpillars returning from an unproductive feeding site (unprofitable) vs. trails marked by fed, successful caterpillars returning from a profitable feeding site (profitable). Significance was assessed with the number of test replicates as degrees of freedom.

Replicate	Larval instar	N	Choice		Total χ^2	p
A			aged trail	new trail		
1-6	L4	60	12	48	29.6	
7-10	L5	40	11	29	9.2	
Total		100			38.8	< 0.001
B			profitable	unprofitable		
1-6	L4	60	47	13	22.0	
7-10	L5	40	33	7	18.8	
Total		100			40.8	< 0.001

When caterpillars were allowed to choose between trails marked by successful, fed caterpillars or trails marked by unsuccessful foragers they significantly preferred the trails of successful foragers (Table 1, χ^2(10df, N=100)=40.8; p<0.001). These results suggest that *E. lanestris* may employ a 2-part trail system, with exploratory and overmarked (recruitment) trails, similar to that found in *M. americanum*. However, there is an important difference in the mechanism of trail establishment between these species. Laboratory observations showed that *E. lanestris* larvae always start foraging en masse, and individual caterpillars do not explore new substrate individually. Rather, in the laboratory, exploration of unfamiliar or new substrate took place by caterpillars in the vanguard proceeding only a few millimeters or centimeters, then turning back and being replaced by another caterpillar that would extend the explored trail slightly further, and so on. Even with large last instar larvae, which would already have begun dispersing in the field, we observed single caterpillars returning from foraging before the start of the next mass foraging in only 2 out of 10 foraging periods observed in detail.

Eighteen out of 20 penultimate instars tested, and all 20 ultimate instars tested, followed trails prepared by swiping the edge of a folded paper across the venter

of the tip of the last abdominal segment (L4: χ^2(1df, N=20)=12.8, p<0.001; L5: χ^2(1df, N=20)=20.0, p<0.001).

Caterpillars of the penultimate instar readily followed trails prepared with 5β-cholestane-3-one at a rate of 10^{-10}g/cm or higher (Table 2). Lower concentrations were not tested as caterpillars obviously reached their physiological limits. With 10^{-10}g/cm pheromone they strongly hesitated to move on any trail and frequently turned back before finally choosing. Last-instar caterpillars were only tested with concentrations of 10^{-9}g/cm trail and also responded positively (χ^2(3df, N=30)=13.6; p<0.01). When allowed to choose between trails applied at concentrations differing by one order of magnitude, caterpillars were only able to distinguish between concentrations if the higher of the two was 10^{-7}g/cm or less (Table 2). In these cases they always preferred the stronger over the weaker trails.

Table 2. Number of *E. lanestris* caterpillars (fourth instar) choosing between trails prepared with different concentrations (in g/cm trail) of 5β-cholestane-3-one or hexane respectively. A concentration of 10^{-10}g/cm corresponds to 2.59×10^{-12}Mol/cm. Results of several replicates with 10-12 individuals each were accumulated. Significance was assessed with the number of test series as degrees of freedom. n.s.: not significant.

Test		Choice (total)		Total N	Total χ^2	df	P
10^{-8}	hexane	27	3	30	20.0	3	< 0.001
10^{-9}	hexane	20	0	20	20.0	2	< 0.001
10^{-10}	hexane	37	13	50	12.86	5	0.025
10^{-6}	10^{-7}	19	11	30	4.0	3	n.s.
10^{-7}	10^{-8}	22	8	20	8.4	3	0.03
10^{-8}	10^{-9}	31	5	36	20.33	3	< 0.001
10^{-9}	10^{-10}	29	3	32	21.13	3	< 0.001

Caterpillars of the third to fifth instar closely followed the S-curved artificial pheromone trails on filter paper, irrespective of mechanical guidelines, by swinging the head left and right, while they failed to cross the filter paper when trails were prepared with hexane only (N=5-10 caterpillars for each series).

The position of a caterpillar in a group exploring a new trail had a significant effect on its walking velocity. Velocity differed significantly between the three behavioral classes (scouts, first five out, and others out) and this was true for both instars tested (2-way-ANOVA; Effects of category: $F(2df, N=98)=88.92$, effects of instar: $F(1df, N=98)=53.00$, $p<0.001$, no interaction effects were found). Figure 2 shows that velocity increases from scouts to "first five out" to "others out". According to their bigger size, fifth instar caterpillars of every category were faster on average than corresponding fourth instars.

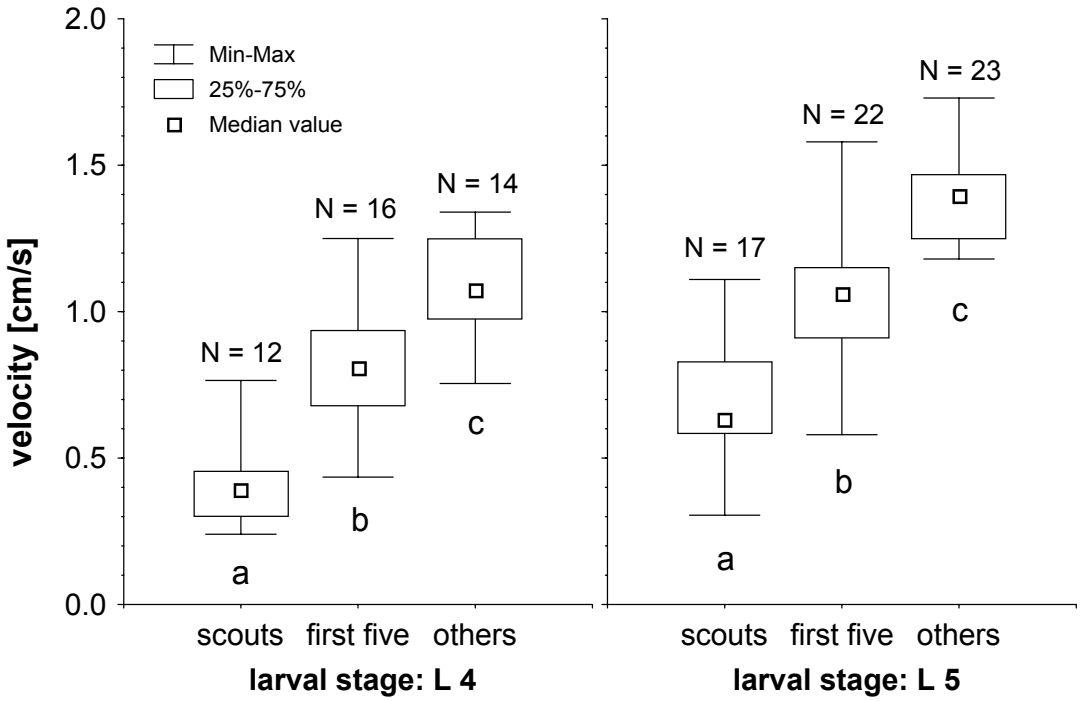

Fig. 2 – Walking velocity [cm/s] of caterpillars (fourth and fifth instar) exploring a new trail, categorized as either "scouts" (not reaching the host), "first five" (reaching the host) or "others" (caterpillars reaching the host later). For calculations medians of all caterpillars measured during the course of one foraging period were used. Sample sizes therefore refer to the number of foraging periods investigated. Boxes marked with different letters differ significantly (Spjotvoll/Stoline test, $p<0.01$, following two-way ANOVA).

Although it was not possible to measure velocity of each caterpillar during each mass foraging period the chronological order of caterpillars measured may serve as an indicator for their position in the group. Although variability was high, Figure 3 shows that mean velocity increased steadily, reaching a saturation point by the 20^{th} caterpillar. By this time the trail seemed to be completely established and there was no further reinforcement.

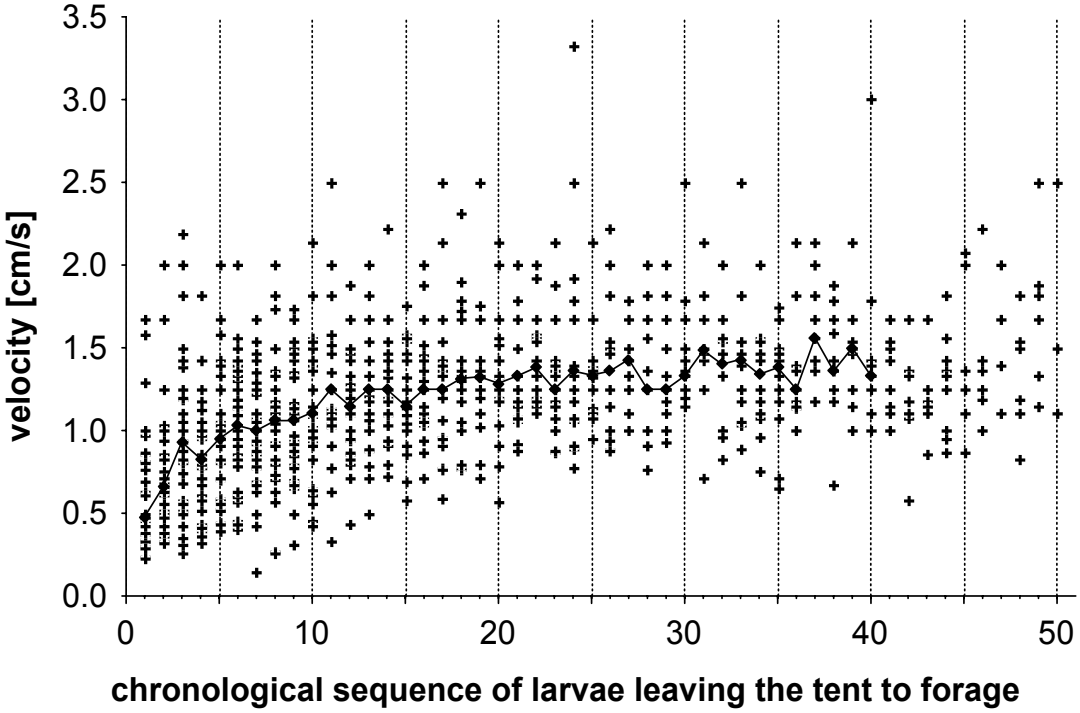

Fig. 3 – Walking velocity [cm/s] of caterpillars exploring a new trail according to the sequence of their registration. The figure shows measured values (**+**) and medians of each position (◆). Median values are connected by a line for better visibility. Sample size is 890 measurements altogether and 11-31 measurements for each position. Medians of the last 10 positions were not calculated because of small sample sizes.

Although these results suggest trails are fully established by the time 20 or more larvae have traversed it to establish a feeding site, we observed that, after feeding, the first five caterpillars subsequently returning to the tent over this trail again moved significantly more slowly than subsequent caterpillars (t-test: t(70df; N=72)=-2,36; p=0.02).

Discussion

The use of trails – although by markedly different means – is a central feature of communication among social caterpillars. Simple trail following systems appear to function in group cohesion, as in the case of patch restricted and nomadic foragers (e.g. Capinera 1980, Fitzgerald & Costa 1986, Roessingh 1990, Fitzgerald 1993a), whereas central place foraging may lend itself to more complex exchange of information between individuals (Fitzgerald & Peterson 1988). Among the approximately 300 Lepidoptera species with social

caterpillars identified to date (Costa & Pierce 1997) only about 11% are central place foragers, and *E. lanestris* is just the fifth of these to be investigated in more detail.

Previous studies had shown that larvae of *E. lanestris* display trail following behavior and use a chemical trail marker (Weyh & Maschwitz 1978), but nothing was known about the complexity of its communication system. Although younger caterpillars of *E. lanestris* deposit copious quantities of silk as they forage, and silk might have been expected to be important for marking trails, our study shows that the attractiveness of a trail is not influenced by the quantity of silk deposited. Trail-following occurs even on artificial trails without any mechanical (silk) guidelines. The same is true for *M. americanum* and *Eucheira socialis*, which readily leave their natural trails for artificial chemical trails (Fitzgerald & Edgerly 1982, Fitzgerald & Underwood 1998b). Last instar *E. lanestris* caterpillars do not deposit any silk while foraging (C. Ruf, unpubl. obs.), yet readily follow natural as well as artificial chemical trails (this study). So, trail following in *E. lanestris* obviously is not bound to silk production, for example as a substrate for pheromone deposition. The coupling of pheromone- and silk-based trails in younger instars may facilitate the recognition of each branch ramification for the smaller larvae, and silk may be expected to improve the hold of the caterpillar on the substrate during foraging.

Caterpillars of *E. lanestris* can discriminate trails of different age, and consistently preferred younger trails. Central-place foraging larval communities are faced with an increasing number of trails radiating from the nest site, and under natural conditions fresh trails have a higher probability of leading to non-exhausted feeding sites. Central-place foraging caterpillars like *E. lanestris* are thus expected to be able to distinguish trails of different ages.

Caterpillars of *E. lanestris* are highly responsive to 5β-cholestane-3-one, a compound of the natural trail phermone of *Malacosoma* spp. yet the threshold of sensitivity is 10 to 100 times higher than that observed in *Malacosoma* spp. (Fitzgerald 1993b, Fitzgerald & Webster 1993). We can presently not determine whether this is due to the experimental conditions. On wooden dowels, a

fraction of the applied pheromone solution may be absorbed and thus will be out of reach of the caterpillar's contact chemoreceptors. Tests with *Malacosoma* have been conducted with small strips of index card where these effects are less likely. Alternatively, *E. lanestris* might use a trail pheromone which could either consist of steroids other than 5β-cholestane-3-one or where 5β-cholestane-3-one is just one compound of the native pheromone blend (cf. Fitzgerald & Webster 1993).

Bioassays of 5β-cholestane-3-one show that *E. lanestris* respond to quantitative increases in artificially-applied pheromone, recognizing only concentrations above a 10^{-10}g/cm threshold in our studies and preferring stronger trails to weaker ones. Thus, during group foraging, the pheromone deposited by each caterpillar increases the probability that subsequent caterpillars recognize and choose the same pathway.

Caterpillars of *Gloveria*, *Malacosoma*, and *Eucheira* all mark trails by dragging the ventral surface of the tip of the abdomen along the substrate, resulting in an altered locomotion cycle. In *E. lanestris* we were not able to explicitly see the marking behavior but the 'wiping' tests, as well as the observation that velocity is reduced during the return of the caterpillars to the tent when trails have already been well established, indicate that marking follows the same pattern as described for those species. Marking may take place only during part of the locomotive cycle as described in *Eucheira*, making it more difficult to observe the details without sophisticated video techniques. Weyh and Maschwitz (1978) suggested that the trail marker of *E. lanestris* might be produced in the spinneret, therefore being a component of the silk. Our results are inconsistent with this hypothesis, because even last instar caterpillars are able to mark trails and recruit to food, but no longer deposit silk.

Recruitment communication has evolved in many eusocial insects (especially ants and termites) and is commonly considered to be a feature of highly sophisticated communication systems. Interestingly, however, recruitment communication frequently does not occur in eusocial species if the distribution of resources does not support such behavioral patterns (Hölldobler & Wilson

1990, Traniello & Robson 1995). Despite their simple demographic structure some social caterpillar colonies exhibit communication systems not inferior to those of some ants and termites (e.g. Fitzgerald & Costa 1999).

The complexity of information encoded in a trail has been a central issue in previous studies on social caterpillars. The trails deposited by larvae of *E. lanestris* obviously encode foraging information, directing unfed group members to profitable feeding sites, as opposed to simply serving as a group cohesive device as found in nomadic and patch restricted foragers. From this point of view the foraging system *of E. lanestris* resembles those of *M. americanum* and *M. neustrium* and is more sophisticated than those of *Eucheira* and *Gloveria*, which do not recruit to food at all or which have only weak recruiting abilities. A comparison of *E. lanestris* and *M. americanum* reveals that the trail following system of the latter differs somewhat in that single scouts may break from the group in search of high quality food (in particular, when exploring old trails), whereas caterpillars of *E. lanestris* only seek food in groups, at least under laboratory conditions. If *E. lanestris* never forages independently under natural conditions, its communication system may have a somewhat reduced capacity for collective flexibility relative to that of *M. americanum* (cf. Fitzgerald & Costa 1999).

Food recruitment in social insects is typically defined as communication by which a successful forager directs nestmates to a resource (Hölldobler & Wilson 1990) but the term is used in a slightly wider sense in social caterpillars (cf. Fitzgerald & Underwood 1998a). Although caterpillars of *E. lanestris* almost never leave the tent alone, as activity is strongly synchronized and individuals forage en masse, they return to the tent if they are not able to find food and are thus likely to detect a trail left by successful foragers and follow it out to a new patch. Depleted feeding sites are thus left in favor of new food finds within the course of a foraging bout (C. Ruf, pers. obs.). We do not know whether *E. lanestris* larvae are also able to discriminate between food patches of different quality, as has been shown for *M. americanum*, where such discrimination abilities contribute to the trail system's complexity (Fitzgerald & Peterson 1983).

It should be emphasized that the studies reported here are laboratory studies, and behavior of the caterpillars may differ to some degree under field conditions. For example, they almost certainly 'recruit' in a stochastic fashion if separate small groups of caterpillars leave the tent at different points for different host plant branches, which we did not permit in our laboratory experiments but which occurs under natural conditions (C. Ruf, pers. obs.).

Central place foraging by itself does not lead to complex foraging systems per se. Fitzgerald and Underwood (1998a) suggested that the patchiness of the food supply and the need to search extensively for food may have led to the evolution of the fine-tuned foraging system of *M. americanum*. Other factors might be important as well. For example, larval development in *E. lanestris*, *M. americanum,* and *M. neustrium* seems to be selected for speed: these caterpillars eclose in early spring, use food that is rich in nitrogen and water (Parry et al. 1998), and show pronounced behavioral as well as metabolic thermoregulation (Knapp & Casey 1986, Joos et al. 1988, Ruf & Fiedler 2000) which allows those species to reduce developmental time. Fast development is further improved through foraging by day and night, a factor not taken into account so far. Diurnal foraging increases the risks of conspicuousness to natural enemies (visually hunting predators, parasitoids), perhaps making it necessary to reduce foraging periods to a minimum. Possibly there are no selective advantages for more slowly developing caterpillars which strictly forage at night – like *Gloveria* and *Eucheira* – to evolve a highly sophisticated foraging system, leading to short efficient foraging bouts and ultimately reducing exposure to natural enemies.

Sociality itself is a rare but widespread phenomenon in the Lepidoptera, occurring in some twenty or more ditrysian families. Although it occurs more frequently in some monophyletic lineages (such as the Lasiocampidae) than in others, which would hint to phylogenetically inherited traits favoring the evolution of sociality ecological factors appear to having shaped the different social systems more strongly (Costa & Pierce 1997). In accordance with this view, our results on the trail communication system of *E. lanestris* revealed that

all its properties well match the ecological requirements of a species exploiting patchily distributed resources in early spring, where thermal constraints strongly favor social life-habits (Ruf & Fiedler 2000). Moreover, our study has shown a number of parallels, but also marked differences, in comparison with the well-known trail communication systems of tent caterpillars of the genus *Malacosoma*. Clearly, much remains to be learned from detailed comparative studies on a range of social caterpillar species to uncover the factors which shape the complexity of their communication systems.

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Chapter 8

The mechanisms of trail-based communication: Trail marking and recruitment

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Abstract

Caterpillars of *Eriogaster lanestris* mark previously unmarked paths by dragging the ventral side of the tip of their last abdominal segment (A10) over the substrate. Crawling without marking is achieved by bobbing up the last abdominal pair of prolegs during the locomotion cycle. Caterpillars only overmark already existing trails as they turn back to the tent, if they had successfully exploited a food patch during the preceding foraging bout, and then continue marking onto the tent itself. Thereby, the attractiveness of trails leading to profitable feeding sites is reinforced. Surface residues wiped off the sternum of A10 from fed or unfed caterpillars onto artificial pathways are undistinguishable for other caterpillars in Y-maze tests. Thus, differences in trail attractiveness that exist between trails connecting the tent with either profitable or exhausted feeding sites must be encoded by the amount of trail marker and are not caused by qualitative differences between exploratory and recruitment trails. Since foraging bouts are strongly synchronized new trails are usually explored by caterpillars in groups and the majority of the tentmates find the new feeding sites before the first successfully foraging caterpillar returns to the tent. Recruitment by individuals as described for the related species *Malacosoma americanum* never occurred in our experiments.

Introduction

Among the huge insect order Lepidoptera, the larvae (=caterpillars) of less than 1% of the known species live gregariously or "socially" at least during earlier parts of their life-cycle (Costa & Pierce 1997). Social caterpillars can be broadly classified as either patch-restricted, nomadic, or central-place (fixed-base) foragers, respectively, depending on their use of resources and the location and the permanence of their resting sites (Fitzgerald & Peterson 1988). It is generally acknowledged that central-place foraging requires the most sophisticated form of communication among the members of the colonies. Parallels and differences in the complexity and effectiveness of trail-based communication systems have been analyzed in five species representing four genera (Fitzgerald 1995 and references therein, Fitzgerald & Underwood 1999a,b, Ruf et al. 2001).

Social caterpillars of *Eriogaster lanestris* pertain to the ca. 30 species of lepidopterous central-place foragers identified so far (Costa & Pierce 1997). With the exception of the last instar these caterpillars produce copious quantities of silk when they move between their stationary tent and their feeding sites on the host plant, leaving trunk trails heavily covered with white filaments of silk. During the course of the caterpillars' development resources become more and more patchily and unpredictably distributed around their home base and numerous ramified trails spread over the whole plant. Thus, the ability to mark trails differently and to clearly distinguish between different trails is crucial for efficient feeding until the end of the caterpillars' social phase of their lives.

Previous studies on tent caterpillars (*Malacosoma* spp.) and other species with central place foraging social caterpillars revealed that the chemical stimulus for trail marking is not intimately associated with the silk. Rather, a pheromone secreted from the tip of the abdomen elicits trail following irrespective of the silk (Fitzgerald & Edgerly 1982, Fitzgerald & Costa 1986, Fitzgerald & Underwood 1998a,b). In contrast to these findings Weyh and Maschwitz (1978) supposed the trail marker of *Eriogaster lanestris* to be a component of the silk produced in the caterpillar's spinneret. However, subsequent studies proved this assumption

to be wrong since caterpillars of *E. lanestris* readily follow silk-free trails, which were attained by wiping the edge of a folded paper between the caterpillars' terminal prolegs (Ruf et al. 2001a). Furthermore, trail-based communication was also observed during the caterpillars' last instar, when they do not produce silk any more. In addition, *E. lanestris* larvae readily follow artificial trails made of 5β-cholestane-3-one, i.e. the same chemical that serves as trail pheromone in *Malacosoma* spp. (Ruf et al. 2001a).

It has been repeatedly shown that orientation on naturally laid trails is facilitated by differences in their attractiveness to the caterpillars (Fitzgerald 1995 and references therein). Obviously, there is a difference between 'exploratory trails' that only serve the relocation of the tent and 'recruitment trails' that indicate trails leading to profitable feeding sites. Trail attractiveness is not a simple function of the number of caterpillars passing the trail (Fitzgerald 1976, Ruf et al. 2001a). Yet, the chemical basis of a bilevel trail system with exploratory trails and more attractive recruitment trails remains unknown for any lepidopteran species. It was suggested that the Eastern tent caterpillar, *Malacosoma americanum*, might use different steroid compounds for differently marking trails (Fitzgerald 1993a). However, the use of one of the two steroids used in these experiments has never been shown for *M. americanum* and consequently remains doubtful. In addition, the source of the trail marker is still unclear and circumstantial evidence suggests that it is probably not localized to a single gland but spread over numerous cells (Fitzgerald 1995). Thus, it appears to be quite unlikely that a caterpillar might be able to control the chemical composition of the trail marker, but direct chemical analyses of the surface residues of unfed and satiated caterpillars have never been conducted. Moreover, a bilevel trail-based communication system might be based upon quantitative characteristics rather than the qualitative chemistry of the trails.

This study aims at answering the following questions:

(1) Where does the trail marker of *E. lanestris* originate from and how does the caterpillar apply it?

(2) Are caterpillars able to modify the trail marker qualitatively or quantitatively according to their physical condition (hungry or satiated)?

(3) How sophisticated is the recruitment system of *Eriogaster lanestris*, i.e. how fast are colonies able to switch from a depleted to a new feeding site?

Material and Methods

Animals: Whole colonies of *Eriogaster lanestris* were maintained in an environmental cabinet at 25°C during the day and 15°C at night (L:D 14:10h). Under these conditions fourth instar caterpillars showed three foraging bouts during the daylight hours with an approximate temporal spacing of four hours, third instar caterpillars had a shorter digestion time (approximately 3½ hours) and could achieve four foraging bouts during daytime. Another 1-2 foraging bouts occurred over night (cf. Ruf & Fiedler 2002).

Preparation of artificial trails with the natural trail marker: In order to obtain defined trails with the natural trail marker, we modified the experimental design of Fitzgerald & Underwood (1998b). A 10 × 6cm sized sheet of paper was folded along both diagonals. Parts of the creases were marked 1cm in one direction and 3cm in the other direction starting from the crossing point (Fig. 1).

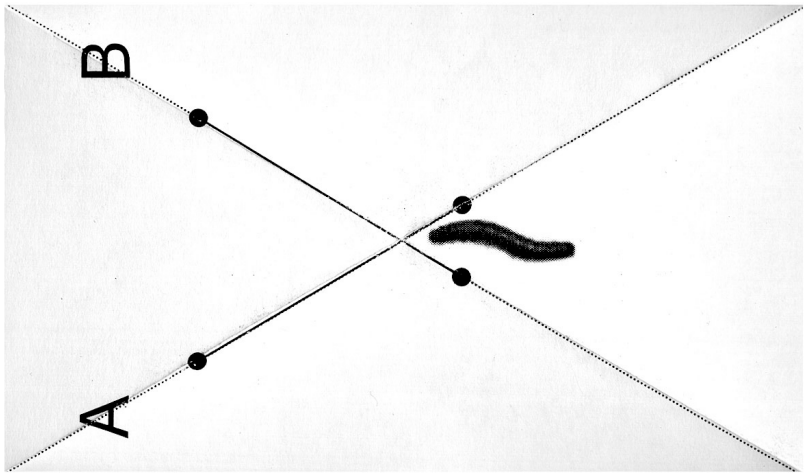

Fig. 1 – Experimental design for assessing the response of caterpillars to qualitatively different trails prepared by wiping off surface residues. Lines between the dots indicate the region, where the marker was applied.

To obtain trails, the venter of the tip of the abdomen of one caterpillar was dragged three times along one of the creases between the markings (Fig. 2, A). The sheet was then unfolded and a caterpillar was placed on the paper with its head between the crossings of the two creases, now building a slight ridge. Swinging its head from side to side the caterpillar touched both creases and could therefore find the prepared trail. A positive response was scored when the caterpillar followed the trail to the mark within three minutes after it had started to search for a trail. Twenty caterpillars of the fourth as well as twenty caterpillars of the fifth instar were tested. As a control, the same experiment was repeated by dragging the folded paper across the caterpillar's body, right before the terminal prolegs (Fig. 2, B). Twenty fourth instar caterpillars were allowed to search for the trail for five minutes each. In all tests the sides of the prepared pathways were changed after half of the experiments to level out potential effects of side preference.

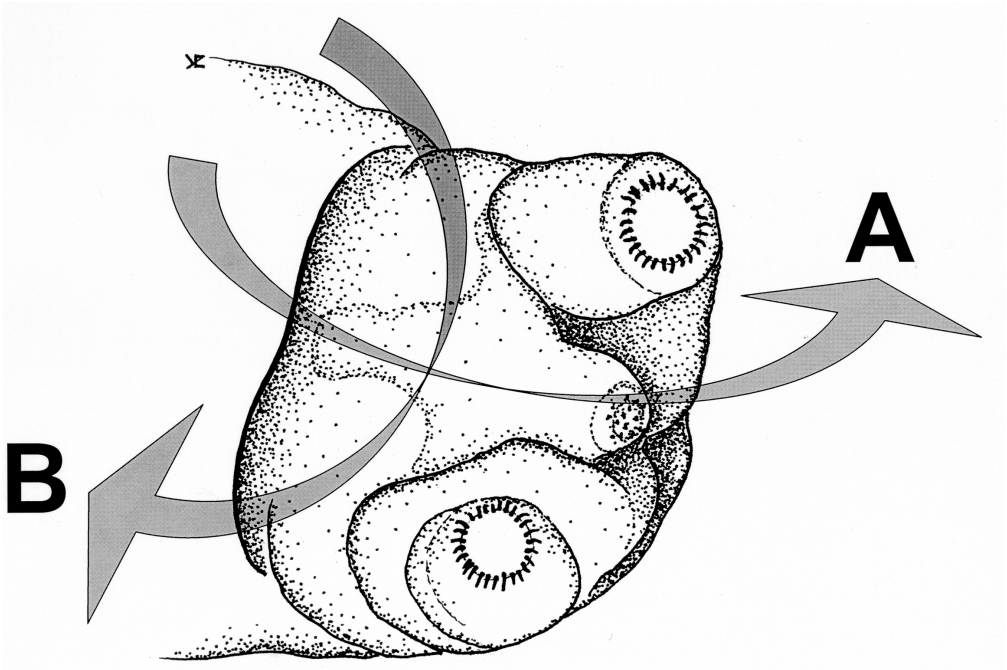

Fig. 2 – Posterior tip of the ventral surface of the abdomen of *E. lanestris*. Arrows show regions from which surface residues were collected as indicated in the text. Drawing courtesy of J. Klein.

Qualitative differences of trails: Orientation of caterpillars must be promoted by qualitative or quantitative differences of trails, either caused by chemical differences of trails marked in different situations (e.g. before or after feeding) or simply by different marking intensity causing differently intense trails. In contrast to natural trail marking it is possible to control for the strength of a trail when wiping surface residues directly off the caterpillar, whereas caterpillars naturally marking trails may voluntarily influence the strength of trail by adding a different amount of pheromone onto the surface. We therefore collected caterpillars which were either on their way to the host plant or on their way back after feeding and collected surface residues as described above, preparing each sheet of paper with the surface residue of one hungry and one satiated caterpillar. Hungry caterpillars taken from a different colony were allowed to choose between the two trails. The test was replicated 40 times.

In two further experiments trails were obtained in the same way as described above but sheets were set aside after preparing one side of the sheet until the next foraging period (i.e. for ca. 4 hours) when the second side of the sheet was prepared. Sheets were then used for the tests at once or aged another time until the next foraging bout. Thus, we created sheets with trails of four hours age difference, once with the older trails having aged for four hours compared to non-aged trails, and alternatively with trails of four and eight hours of age, respectively. Test caterpillars were thus confronted with new trails and trails from the directly preceding foraging bout, or with trails from the two preceding foraging bouts respectively, corresponding to natural conditions.

Marking behavior: Movements of caterpillars during locomotion were recorded with a video camera. Representative sections of the video frames were digitalized (Dazzle Movie Star: Digital video creator II) and analyzed on the basis of single pictures (Action Image Systems Technology: Video capture utility für Windows 95). Locomotion patterns were drawn by firstly copying individual pictures one by one in single file into a word processing program (Microsoft Word 2000) and by then redrawing the outlines of the caterpillars for better clarity.

Recruitment experiments: For analyzing recruitment abilities of *Eriogaster lanestris* we slightly modified the experimental design used by Fitzgerald & Underwood (1998a,b) (Fig. 3). During the first foraging bout of each experimental day after the light had turned on and temperature had been elevated from 15°C to 25°C caterpillars got access to only one wooden bridge leading to a bunch of blackthorn (*Prunus spinosa* L., Rosaceae, a major host plant of *E. lanestris*) twigs. The bridge was attached to a stem section, which was retained during the whole series of experiments with the exception of the very end of the stem which was replaced prior to each new test. In the subsequent foraging bout a new bridge was attached to the stem section, now forming a Y. A bunch of host plants was offered at the end of the new bridge whereas a bare branch was attached to the end of the old trail. During the third foraging period all settings from the foregoing foraging period were retained.

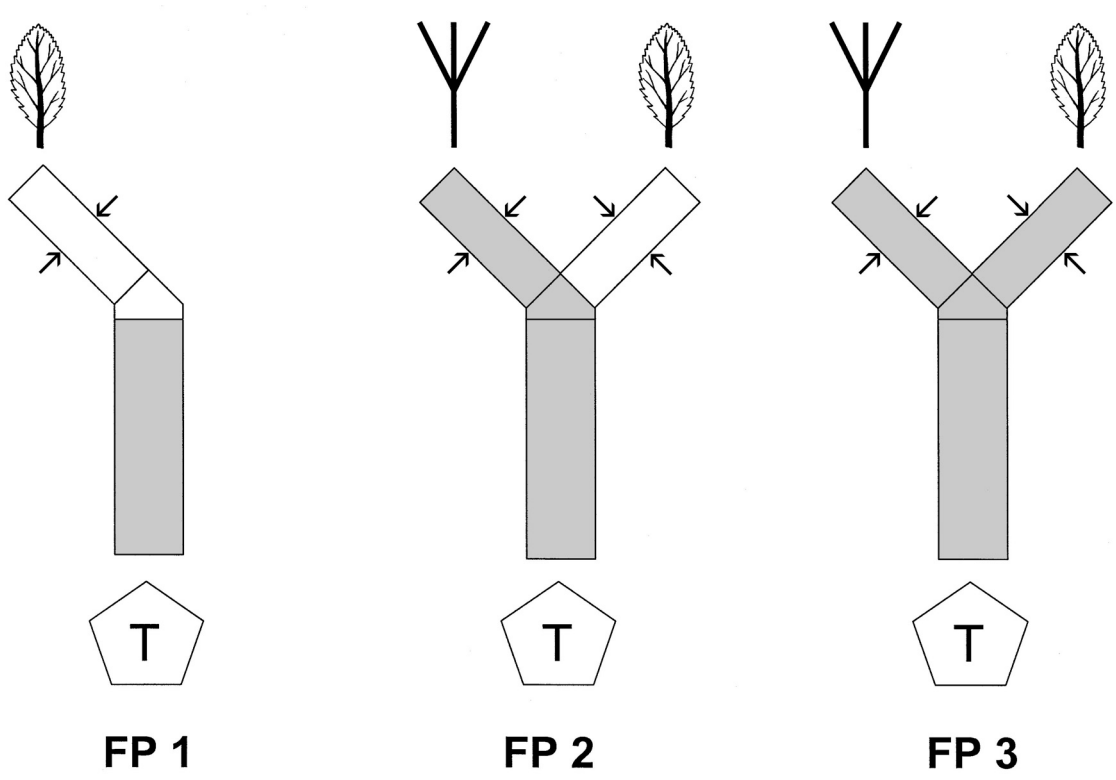

Fig. 3 – Experimental design for analyzing recruitment abilities of *Eriogaster lanestris* caterpillars. Transparent bar: new rod; gray bar: marked trail. T=tent; FP=foraging period. Arrows indicate position of light barriers. The leaf symbol denotes a bunch of the host plant, the other symbol a bare branch.

The decision making of caterpillars was recorded with the help of light barriers placed at both sides of the arms of the Y (cf. Ruf et al. 2001b). The counts of the light barriers could not be used as an exact measurement for the number of caterpillars using a trail per minute, because caterpillars that search intensively for a trail move hesitantly over the bridges and may release several counts by moving their head back and forth. Nevertheless, the counts serve as a reliable relative measurement for the attractiveness of a trail for the caterpillars.

During the second foraging bout it was noted when the first caterpillar reached the plant and when the first satiated caterpillar left the plant. Ten colonies were tested on 2-3 consecutive days.

Conditions of trail marking: It is well known from other social caterpillars, especially those of the genus *Malacosoma*, that trail marking consists of two steps: marking behavior on new trails and overmarking when caterpillars return to the tent after feeding. In order to determine under which conditions caterpillars of *E. lanestris* mark or overmark trails we used the experimental setup described above but with only one section attached to the stem. We observed fourth instar caterpillars passing the established stem section (abbreviation: “establ.”) and a previously unmarked branch (“new”) and noted whether the caterpillars did, or did not, show marking behavior. We used three categories to define the marking process: “marking” (clear, intense marking), “discontinuous marking” (caterpillar changed between marking and not marking), and “no marking”. We noted the direction of the caterpillars and differentiated between caterpillars leaving the tent, and caterpillars returning to the tent without feeding or after successful feeding, respectively (“unfed outbound”, “unfed back”, “fed inbound”). Altogether 10 replicates using four colonies were recorded. During each foraging bout 25 caterpillars of each category were registered with the exception of the category ‘unfed back’ where only 15-17 caterpillars were registered in four of 10 cases.

Results

Choice tests: Caterpillars readily followed trails prepared from surface residues gained from the ventral side of the last abdominal segment between the terminal prolegs. Trails prepared from penultimate and ultimate instar caterpillars were equally accepted (Table 1). In contrast, the majority of caterpillars was not able to find a trail when the surface residues had been wiped off anterior to the last abdominal prolegs.

Table 1. Results of trail marking tests (data summarized from all replicates of one experiment, but significance was assessed with the number of test replicates as degrees of freedom). Data in the first two rows from Ruf et al. (2001a). All experiments done with fourth instar caterpillars unless otherwise stated. n.s. = not significant.

Experiment	Choice		N	χ^2	df	p
Wiping between last abdominal prolegs (L4)	Marked trail: 18	Unmarked trail: 2	20	12.80	1	<0.001
Wiping between last abdominal prolegs (L5)	Marked trail: 20	Unmarked trail: 0	20	20.00	1	<0.001
Wiping before last abdominal prolegs	Marked trail: 9	Unmarked or no trail: 21	30	4.80	2	n.s.
Satiated vs. hungry caterpillars	Satiated: 22	Hungry: 18	40	2.78	3	n.s.
Trail difference 4h, Exp. 1	New (0h): 26	Old (4h): 4	30	41.39	3	<0.0001
Trail difference 4h, Exp. 2	Newer (4h): 35	Older (8h): 15	50	8.63	3	<0.05

Caterpillars could not differentiate between trails either obtained from satiated or hungry larvae, respectively. If caterpillars were to choose between new trails and trails which had aged for four hours they highly significantly opted for the new trails. If caterpillars were offered trails that had aged for either four or eight hours they still significantly preferred the younger trails but the difference was less pronounced and caterpillars examined both trails several minutes before they chose one of them.

Marking behavior: Observations of caterpillars moving between their tent and their host plant revealed two distinct locomotive patterns. Figure 4 (left side) shows a typical motor pattern of a caterpillar marking a trail: Starting with the

caterpillar being stretched to its maximum length, with all prolegs attached to the substrate, locomotion starts with the thoracic legs dragging the body forward. During the concomitant contraction of the body the pair of terminal prolegs is dragged in the direction of crawling (Fig. 4: a1-a5). It is noticeable that during this part of the locomotory cycle the terminal prolegs are never lifted, as it is common in caterpillars, but always remain on the substrate. To achieve a maximum compression of the body the abdominal prolegs A5 and A6 are lifted. As soon as the pair of terminal prolegs is re-anchored (Fig. 4: a5) the remaining abdominal prolegs are planted and lifted and planted again (progressing from A6 to A3), resulting in a body wave moving from back to the front until the caterpillar is totally stretched again, fixed with all prolegs to the substrate, and a new locomotive cycle starts (a5-a7).

In contrast to this locomotive pattern *E. lanestris* also shows another way of movement which can particularly be seen during faster locomotion (Fig. 4, right side). During the phase of re-planting the foremost abdominal prolegs which is exactly the moment when the caterpillar becomes maximally sprawled (b1-b2), the pair of terminal prolegs rapidly bobs up (Fig. 4, marked with arrow). It is conspicuous that this movement does not yet serve the contraction of the body because the terminal prolegs are not yet anchored immediately after they have been re-planted. Instead, the contraction of the body only starts afterwards (b3-b6) and the subsequent body wave occurs in exactly the same way as explained above. During the contraction of the body the terminal prolegs are set more vertically than during the locomotor pattern described above when they are more straddled which brings the region between the terminal prolegs in better contact with the substrate. However, differences between marking and non-marking are weaker and less obvious in *E. lanestris* compared to the marking mode described for other lepidopteran species.

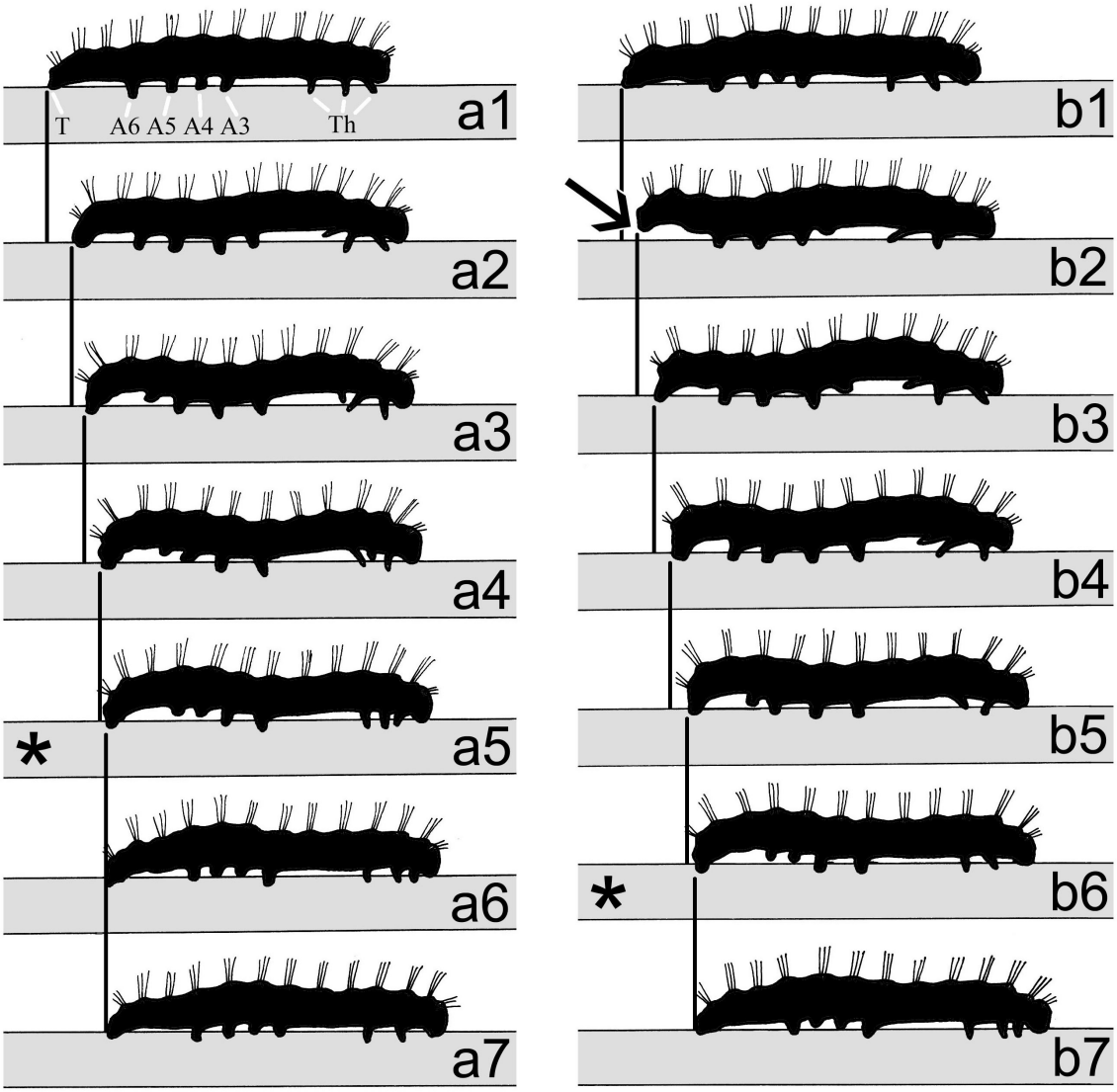

Fig. 4 – Patterns of locomotion in caterpillars of *Eriogaster lanestris* (one locomotor cyle). **T** terminal prolegs, **A3-A6** abdominal prolegs, **Th** thoracic legs. Patterns differ by the way how the terminal prolegs are moved. During marking (left) the terminal prolegs almost always contact the substrate and marking is supposed to occur particularly strongly during a1, a6, a7. Non-marking caterpillars (right) must bob up the terminal prolegs to avoid marking during the phase when the body is maximally stretched (see arrow). Asterisks mark the forward moment when the body is anchored by the terminal prolegs (plantae everted). Vertical lines indicate the progression of the terminal prolegs during locomotion. Caterpillar on the right is slightly bigger and faster than caterpillar on the left. Total duration of one cycle: ca. 0.3s (at room temperature).

We assume that the only purpose of this unusual locomotor behavior is to prevent the sternum of the last abdominal segment from contacting the substrate. Thus, larvae of *Eriogaster lanestris* commonly mark their trails during their ‘normal’ movements particularly in the moment when the caterpillar is

maximally sprawled (Fig. 4: a1, a6, a7), and must actively avoid contacting the substrate in order to crawl without laying a trail. The trail substance can thus be expected to be applied more or less continuously but is probably not equally highly concentrated over the course of the entire trail.

Recruitment: Individual caterpillars that encountered a new, yet unmarked trail usually turned round and returned to the tent or made only little progress. Thus, new trails were only slowly established as a result of marking activities of multiple caterpillars in a group. During the second foraging period (FP2, Fig. 3, middle) caterpillars intensively searched for food on the bare branch, to which they were led by the trail marked during FP1. The new site of the plant at the other arm of the Y was only found after an average of 31±12 minutes (N=26, pooled data from 10 colonies) after the beginning of the foraging period. Since individual activity is very well synchronized in *E. lanestris* and all caterpillars participated collectively in the search for new food, 41.5±9.2% of the signals registered by the light barriers aside the new rod during the second foraging period appeared before the first satiated caterpillar returned from feeding at the new site. The first caterpillar which reached the new plant required 11.2±2.2 minutes (N=26) to crawl on the branch to a suitable feeding site, feeding, and returning to the rod. Since each caterpillar passed the light barrier at least twice during one foraging period, this means that about 83% of the caterpillars finally reached the plant during FP2 prior to the return of the first satiated caterpillar to the tent (cf. Fig. 5). Thus, immediate recruitment of food-searching caterpillars by fed individuals or small groups never took place in our experiments.

During the third foraging period (FP3, Fig. 3, right) only few caterpillars chose the old trail but occasionally the old trail was still attractive and preferred over the new trail during the first half of the foraging period (Table 2). It is noteworthy that such less efficient recruitment was mainly shown in those colonies that were held at lower temperatures some weeks before the experiment and recruitment became more efficient when the experiment was repeated on the consecutive days. Overall, only 2.1% of the signals were registered on the old trail.

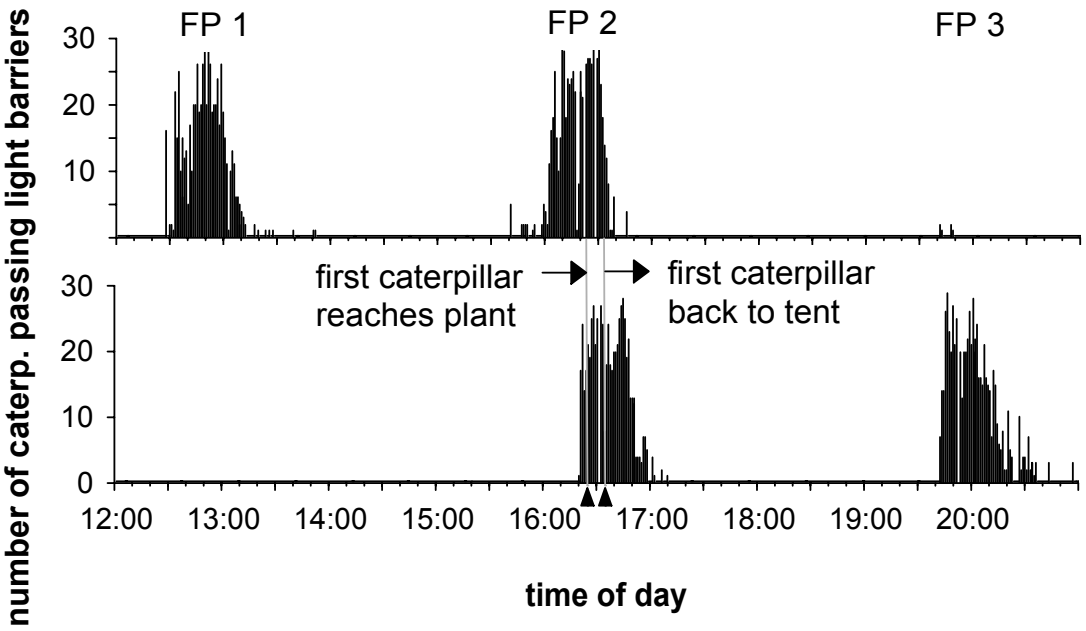

Fig. 5 – Exemplary results of the Y-test over a period of three foraging bouts. Upper graph: first offered rod. Lower graph: secondary offered rod. FP = foraging period. The majority of caterpillars chose the new rod clearly before the first satiated caterpillar crawls back to the tent.

Marking intensity: When leaving the tent, only few caterpillars marked the established trail near the tent but they started to mark heavily when they encountered the previously unmarked or yet only weakly established trail (Fig. 6). If caterpillars turned back without having found the food, the majority did not mark at all or only marked occasionally. If caterpillars returned to the tent after feeding they strongly marked both, the previously newly marked trail and the well established trail and continued marking onto the tent itself.

Table 2. Proportion of signals on older trails [%] ('Prop.') during the third foraging period (FP3 = succeeding foraging bout after food was switched from the end of the old rod to the end of the new branch). Caterpillars of colonies 4-8 were held at 15°C prior to the experiments. *** p<0.0001, n.s. not significant, o.p. opposite preference (p<0.0001, for old trail). χ^2-tests (with 1 degree of freedom) are based on actual counts on both rods compared to the null hypothesis of equal distribution of the counts.

Colony	Replicate	Entire FP			First half of FP			Second half of FP		
		Prop.	χ^2	p	Prop.	χ^2	p	Prop.	χ^2	p
1	1	2.3	587.4	***	3.9	237.7	***	1.1	350.2	***
1	2	0.8	247.1	***	0.8	233.1	***	0.0	113.0	***
1	3	5.2	354.8	***	7.7	215.0	***	0.0	142.0	***
2	1	0.8	757.2	***	0.9	681.2	***	0.0	56.0	***
2	2	22.4	316.8	***	27.7	166.5	***	0.0	200.0	***
2	3	0.0	768.0	***	0.0	520.0	***	0.0	248.0	***
3	1	0.0	590.0	***	0.0	483.0	***	0.0	107.0	***
3	2	0.0	577.0	***	0.0	327.0	***	0.0	250.0	***
4	1	65.1	227.7	o.p.	77.9	583.2	o.p.	27.1	132.6	***
4	2	26.2	102.8	***	37.5	19.3	***	2.7	131.4	***
5	1	48.4	1.8	n.s.	62.5	80.8	o.p.	17.4	248.1	***
5	2	17.0	236.5	***	23.5	110.4	***	0.0	150.0	***
5	3	0.9	562.2	***	1.4	325.3	***	0.0	237.0	***
6	1	30.4	202.0	***	37.3	65.6	***	6.4	223.9	***
6	2	7.5	563.3	***	10.7	333.9	***	0.0	237.0	***
6	3	11.8	636.0	***	15.6	392.4	***	0.0	263.0	***
7	1	30.3	95.7	***	46.9	1.6	n.s.	0.0	218.0	***
7	2	0.0	540.0	***	0.0	356.0	***	0.0	184.0	***
7	3	0.0	548.0	***	0.0	330.0	***	0.0	218.0	***
8	1	0.9	426.1	***	1.4	265.2	***	0.0	161.0	***
8	2	0.0	436.0	***	0.0	248.0	***	0.0	188.0	***
8	3	0.5	358.0	***	0.9	218.0	***	0.5	146.0	***
9	1	0.2	559.0	***	0.4	267.0	***	0.0	292.0	***
9	2	2.5	583.6	***	4.1	311.4	***	0.4	273.0	***
10	1	1.8	807.2	***	2.8	508.8	***	0.0	299.0	***
10	2	6.3	364.5	***	11.0	165.2	***	0.0	205.0	***
Median		2.1			3.4			0.0		

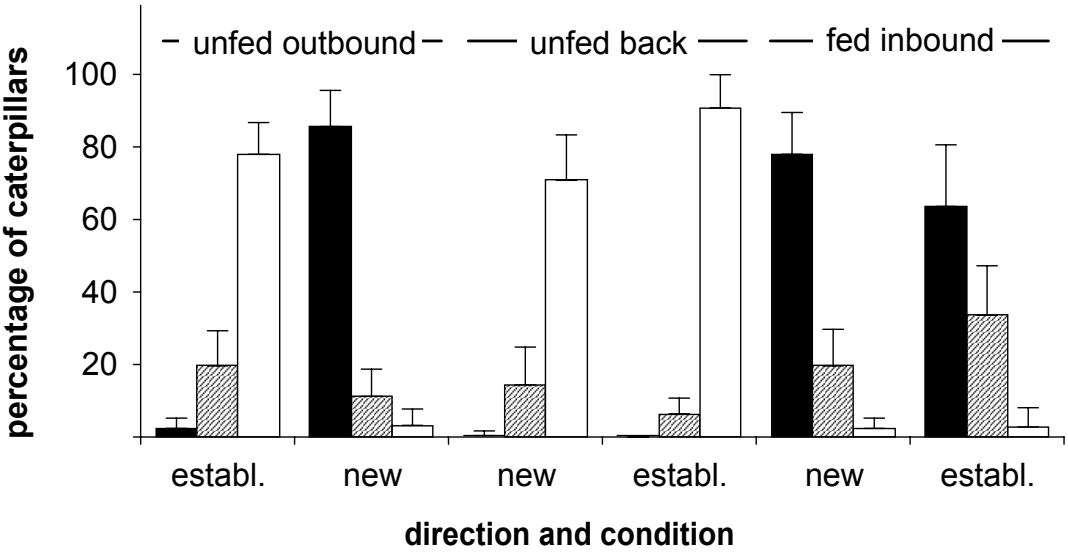

Fig. 6 – Behavior of caterpillars passing previously unmarked ('new') or well established ('establ.') trails under different conditions. Black bars: marking, hatched bars: discontinuous marking, white bars: no marking. Pooled data from all colonies and replicates. Marking intensity is significantly higher in unfed outbound caterpillars on new compared to established trails, and fed caterpillars returning to the tent mark significantly more intensively compared to unfed caterpillars turning back on new and established trails, respectively (pairwise t-tests, all $p<0.0001$ after Bonferroni correction, arc sine-squareroot transformed data). Other, non-relevant combinations were not tested.

Discussion

Evolution of trail marking modes in caterpillars

The mode how caterpillars mark trails is quite variable across species, even within the moth family Lasiocampidae. Solitary caterpillars of *Dendrolimus pini* mark trails by dragging the terminal prolegs passively behind, keeping the plantae of the terminal prolegs completely retracted (Ruf & Fiedler 2000).

Tent-building caterpillars of *Malacosoma americanum, M. disstria, M. neustria* and of a non-specified species in the genus *Gloveria* have all been reported to mark trails by dragging the tip of the abdomen against the substrate while straddling the terminal prolegs during a whole locomotive cycle. This is believed to produce a continuous trail (Fitzgerald & Edgerly 1982, Fitzgerald & Underwood 1998a). In contrast, *Eucheira socialis* (Pieridae) uses a distinctly different pattern, marking during only part of a locomotive cycle when the larva

stretches its body and presses the ventral surface of the tip of its abdomen down. These point-like contacts with the substrate result in the deposition of a discontinuous trail (Fitzgerald & Underwood 1998b). Whether *Eriogaster lanestris* deposits its trail marker discontinuously, since abrading of the ventral side of the last abdominal segment should be maximal when the caterpillar is fully stretched and might be very low during other parts of the locomotive cycle, or if deposition occurs more or less continuously, since the up and down movements of the terminal segments probably result in smearing of the marker, remains unknown. However, a discontinuous application of the marker should not influence the functionality of trails since a caterpillar only progresses a fourth or a fifth part of its total body length during one locomotive cycle, which is a range that can easily be scanned by a subsequent caterpillar. Moreover, since trail-marking in *E. lanestris* only occurs as a collective behavior of multiple individuals which forage synchronously en masse, even discontinuous individual trail marks will always sum up to a rather continuous, graded trail.

Despite their different ways of marking all species use secretory sites at the tip of the abdomen. It has been hypothesized that marking with the tip of the abdomen derived from advance-withdrawal conflict behavior associated with movement into unmarked terrain (Fitzgerald & Underwood 1998a,b). By strongly stretching the body while scanning its environment, the ventral surface of the tip of the abdomen necessarily contacts the substrate, and this might have set the stage for the evolutionary conversion of the sternal region of the 10^{th} abdominal segment to a trail-marking organ. If this evolutionary scenario is correct, marking behavior in *Eriogaster lanestris* as well as in *Eucheira socialis* is still more motivationally linked to conflict behavior whereas in *Malacosoma* spp. and *Gloveria* sp. trail marking is more emancipated from its motivational basis in conflict (Fitzgerald & Underwood 1998b). In *E. lanestris* larvae the sternal region of A10 does not carry any noticeable glandular openings (SEM inspection, M. Obermayer, pers. comm.). In addition, histological sections did not reveal distinct clumps groups of enlarged glandular epidermis cells (M. Obermayer, pers. comm.). Therefore, the most likely source of the trail pheromone are individual secretory cells that occur interspersed in the

epidermis of the region of the integument that proved to carry biologically active surface residues.

Marking with glands situated at the tip of the abdomen is also the predominant mode of marking in ants and termites (Hölldobler & Wilson 1990). Nevertheless, glands producing the trail markers are morphologically very diverse in different families or genera in ants and must have evolved independently multiple times. In analogy, the variation of trail marking behaviors that can be observed across the Lepidoptera suggests that trail marking has evolved independently a number of times. This view is strongly supported by the scattered, and overall rare, occurrence of trail-marking in solitary lepidoperan caterpillars (Weyh & Maschwitz 1982, Tsubaki & Kitching 1986, Ruf & Fiedler 2000), as well as by the sporadic distribution of species with trail-marking social larvae across the higher clades of Lepidoptera (Costa & Pierce 1997). Even within the family Lasiocampidae phylogenetic evidence suggests that social life-styles and trail marking have evolved more than once (Regier et al. 2000).

It is noteworthy that social caterpillars that forage in a 'patch-restricted' manner or by tandem trail-following in a nomadic way also use pheromones for trail marking, but these chemical are not produced at a secretion site at the posterior end of the body (Capinera 1980, Roessingh 1990, Fitzgerald 1993b). Instead, in these instances the pheromone is part of the silk and the mechanical cues of the silk alone can be sufficient to elicit trail following behavior. In patch-restricted foragers the pheromone primarily serves for maintaining group cohesion as well as for marking the foraging arena. Only rarely are silk-based trail pheromones used to reassemble the cohort at a new foraging site. Similarly, tandem trail-following species might also use the pheromone for arena marking only, since cohesion of group members during migration is achieved by direct body contact of the caterpillars. Thus, in these species with putatively less advanced social systems there has been no selection for a marking system that is independent of the ubiquitous deposition of silk along paths walked by a caterpillar.

Reinforcement of trails by overmarking

In central-place foraging species, there is a need for regulating when and how an individual participates in marking trails. Otherwise, the information content of a trail system would severely be affected (e.g. in a 'chaotic' network of individual trails). Social central-place foraging lepidopterous larvae exhibit a range of behavioral strategies in this respect. Caterpillars of *Eucheira socialis* only mark non-established trails but mark little if at all, when they pass over previously marked trails or when they return to their tent after feeding. Obviously, initial trail marking is sufficient to bring the branch to a threshold level of acceptability after which caterpillars do no longer reinforce the trail (Fitzgerald & Underwood 1998b). In *Gloveria* sp. caterpillars mark heavily on previously unmarked trails and overmark newly established trails after feeding, but mark little if they move onto the well established portions of their trail system and on the surface of the nest (Fitzgerald & Underwood 1998a). In contrast, Eastern tent caterpillars, *Malacosoma americanum*, overmark existing trails and continue marking up to the surface of the tent (Fitzgerald 1995).

The trail marking behavior of *Eriogaster lanestris* mostly resembles that of *Gloveria* sp. because individuals do not recruit tent-mates to food. Nevertheless, *E. lanestris* caterpillars continue marking on the well established trail near the tent and the tent itself.

Under natural conditions, tents of *E. lanestris* are predominantly found on the sun-exposed, south-facing outmost branches or twigs of host plant trees (Ebert 1994, C. Ruf, pers. obs.). Thus, tents often have only one main trail which has to be passed by the caterpillars during each foraging bout and which is often strongly coated with silk. Nevertheless, overmarking of new and old trails assures that newly established trails become more attractive than existing but abandoned old ones and that trunk trails leading to the new trails will continue to be attractive during the subsequent foraging periods.

The mechanisms of recruitment

E. lanestris possesses a bilevel trail system that can be functionally categorized as 'exploratory' and 'recruitment' trails (cf. Ruf et al. 2001a). 'Exploratory trails'

are laid whenever a larva or a group of larvae walk over previously unmarked pathways and enable the caterpillars to relocate their tent, even after unsuccessful foraging. These trails are weakly attractive when the larvae leave the tent for the next foraging period some hours later. 'Recruitment trails', in contrast, are laid when caterpillars return from a profitable feeding site. These trails direct the caterpillars to this same site again at the onset of the next foraging period, i.e. result in a temporally shifted recruitment effect (Ruf et al. 2001). Hence, there is no recruitment in a more narrow sense by individual caterpillars and within one foraging period the effect of recruitment becomes only operative (if at all) for few lately foraging, less synchronized caterpillars. In our experiments, individuals did hardly cross previously unmarked sections and colony activity was so tightly synchronized that the majority of caterpillars found the new trail leading to a new profitable feeding site before the first caterpillars left the plant after feeding. Although the arms of the Y were short in our experiments we assume that even longer pathways would not have influenced the results since caterpillars move quickly on established trails. Small L3-caterpillars (mean velocity = 0.53±0.08m/min at 25°C, N=25) would be able to walk nearly 6 meters within the time foraging requires for the first caterpillars to reach the plant, feed, and return to the trunk trail. For fourth and fifth instars, the respective range would even amount to over 7m or over 9m, respectively, based on the velocity data from Ruf et al. (2001a), even disregarding the longer feeding times of larger caterpillars. Thus, most caterpillars would have found the new feeding site within the feeding time of the first caterpillars even if the distances between profitable and depleted feeding sites had been chosen longer.

Obviously, the reinforcement of an exploratory trail into a recruitment trail can only be achieved by numerous caterpillars overmarking the trail after feeding. As a consequence, the recruitment effect of the trail is temporally shifted to the beginning of the consecutive foraging bout.

With regard to the complexity of trail marking and collective flexibility, *E. lanestris*, ranges between *Gloveria* sp. and *M. americanum* (see Table 3). It was argued that the necessity to recruit precisely to food, as it is the case in *M.*

americanum, arises only if caterpillars strongly depend on the youngest leaves as food which are patchily distributed over the whole plant (Fitzgerald & Underwood 1998a). In blackthorns, the youngest terminal shoots do not seem to provide qualitatively better food for the caterpillars and are not fed upon preferentially (C. Ruf, pers. obs.). Thus, the necessity to evolve recruitment by individually foraging larvae may not have arisen in *E. lanestris*.

Table 3. Comparison of foraging and trail marking behavior of four central-place foraging social caterpillar species.

	Malacosoma americanum[a]	*Eriogaster lanestris*[b]	*Gloveria* sp.[c]	*Eucheira socialis*[d]
Marking new branches	+	+	+	+
Marking on turn-back when searching food	-	-	-	+
Marking on newly established trails after feeding	+	+	+	-
Marking on older trails leading back to the tent and onto tent after feeding	+	+	-	-
Recruitment to food	strong	medium	weak	none
Dependency on young leaves	+	-	-	-

a Fitzgerald 1995, b this study, c Fitzgerald & Underwood 1998a, d Fitzgerald & Underwood 1998b

It was suggested that caterpillars of *M. americanum* are able to modify exploratory and recruitment trails by using different pheromones when overmarking trails after feeding (Fitzgerald 1993a). We propose that recruitment in *E. lanestris* can be achieved by the opposing effects of temporally changing trail concentrations only, resulting from overmarking on one the hand and trail aging on the other, and need not be further modified by different trail substances (cf. Fig. 7).

Caterpillars of *E. lanestris* show trail following behavior on artificial trails made of 5β-cholestane-3-one (Ruf et al. 2001a). Thus, it is likely that this substance or a slightly different steroid is the active component of the caterpillars' trail marker. Caterpillars are highly capable of discriminating between artificial trails with different concentrations of this steroid. This dose-sensitivity is necessary

and sufficient for the caterpillars to distinguish between trails marked by different numbers of tentmates, marked with different intensity, or where the active substance has been partially lost during aging of the trail. The mechanism of aging is yet unclear. In ants trail pheromones are often quite volatile substances resulting in short-lived trails that are quickly abandoned if they are not reinforced (Hölldobler & Wilson 1990). In contrast, steroids are larger molecules that have a much lower vapor pressure and are thus not volatile. Moreover, derivates of cholesterol such as 5β-cholestane-3-one are very stable and are thus not expected to degrade within hours (K. Seifert, pers. comm.).

Assuming that quantitative differences between trails are sufficient, the processes during recruitment could be described as follows (numeration from Fig. 7): At the beginning of the foraging bout when food has been switched to the new side (2) caterpillars first follow the well established trail from the earlier foraging period and only choose the new branch if they repeatedly fail to find food (3), a pattern which has been shown experimentally for *M. americanum* (Peterson & Fitzgerald 1991). These exploratory trails are only marked up to a low threshold level that enables the caterpillars to perceive the trail substances quickly (4). Simultaneously, the old trail has lost intensity due to aging because it is not reinforced although many caterpillars pass the trail. Only if caterpillars are satiated they overmark the already existing trail to a much higher threshold level. At the end of a foraging period the new trail leading to the profitable site is consequently much more attractive due to its higher quantity of pheromone (through overmarking), whereas the old trail becomes weaker due to aging (5). This relative difference persists to the next foraging bout (6) and suffices to direct the larvae to the new feeding site. As a consequence, a recruitment effect seems feasible without the existence of a second pheromone.

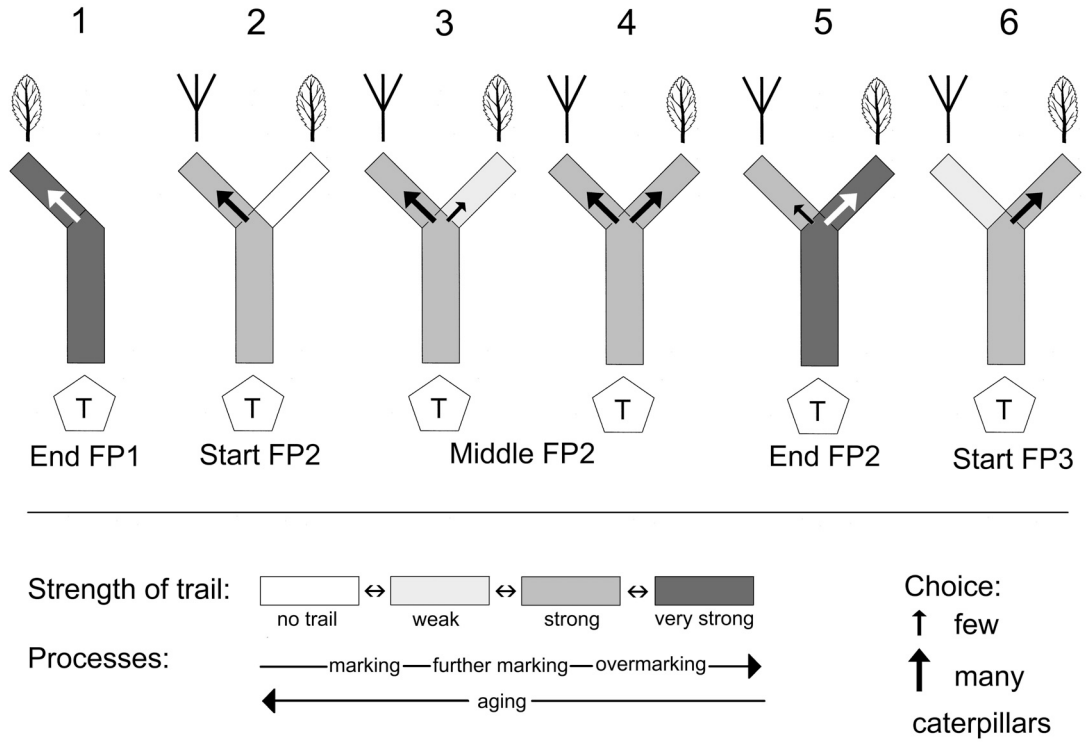

Fig. 7 – Proposed mechanism of recruitment effect and decision-making in foraging *E. lanestris* larvae based on quantitative effects of trail quality alone. Marking increases, aging decreases the strength and therefore the attractiveness of the trails. Switching from an old to a new trail occurs because the old trail is no more overmarked in the second foraging period (FP) if no food is detected. As soon as the new trail is heavily overmarked by fed caterpillars it becomes more attractive than the old trail that has aged since the preceding foraging period.

In line with this argumentation, surface residues of either fed or unfed caterpillars proved to be indistinguishable for the caterpillars in our experiments. Moreover, the observation that larvae of *Eriogaster lanestris* must actively avoid marking by bobbing up the terminal prolegs is a further hint that they are not able to actively control the amount or quality of pheromone deposited on the substrate during contact. Instead, the secretory cells are supposed to produce pheromone continuously and the caterpillar determines whether the pheromone is wiped off or not by controlling the posture of its terminal prolegs.

In ants, where trail systems have been studied much more intensively, trail-based communication can be based on qualitatively different orientation trails, recruitment trails or even long-lasting permanent trails marked with different pheromones produced in different glands (e.g. Attygalle et al. 1988, 1991,

Quinet & Pasteels 1991, Kohl et al. 2001). For other ant species, computer simulations suggest that quantitative differences alone in combination with behavioral thresholds might be responsible for the trail preferences (Goss et al. 1990). Unfortunately, there is no experimental proof yet for the existence of a trail system based solely on pheromone quantities as a consequence of limited possibilities of detecting and quantifying small quantities of pheromones. New studies on the chemical composition of the trail markers of *M. americanum* and *E. lanestris* directly extracted from the trails are necessary to finally address this question.

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Chapter 9

Mechanisms for and consequences of behavioral synchronicity in *Eriogaster lanestris*

Abstract

Caterpillars of *Eriogaster lanestris* exhibit a high level of synchronization of their individual activity patterns. For example, foraging is usually so tightly synchronized across the colony that the majority of caterpillars leaves the tent as a group in search of food within a few minutes. Although synchronization requires communication between tentmates at the onset of each foraging bout, the mechanistic basis of this exchange of information has not yet been determined for any social caterpillar.

This study tests the hypothesis that synchronicity of the caterpillars is related to substrate vibrations arising from the movements of the caterpillars on the tent. Data were acquired by long-term monitoring of tent vibrations with a laser Doppler vibrometer and parallel automatic monitoring of foraging patterns of the caterpillars via light barriers.

Tent vibrations increased and decreased irregularly during the resting phases and frequently, but briefly exceeded a permanent resting level of vibrations (background noise). The final building up of vibrations before the start of a foraging bout lasted on average 19 minutes at 25°C. The start of a foraging bout precisely coincided with the time when the increase of vibrations (i.e. the increase of restlessness among tent mates) was at its maximum.

Group size had a significant impact on synchronicity of the larvae. Caterpillars in large colonies are better able to synchronize their activities and develop faster even though environmental conditions (temperature, light regime, food availability) were kept identical.

Introduction

One of the most striking features of large assemblages of animals is probably behavioral synchronicity among hundreds or even thousands of individuals. Synchronized activity patterns are known from a broad range of animal species like fish, mammals and many insects (for an overview see: Krause & Ruxton 2002). In a recent study Conradt & Roper (2000) focusing on ruminants suggested that many of the advantages of being in a group only emerge if animals perform a given activity (e.g. foraging or resting) synchronously. However, synchronizing activities is assumed to be costly for group members because it may require the postponement of activities despite individual needs. Thus, it is predicted that groups are more stable if they are homogeneous (e.g. with respect to size and age, Conradt & Roper 2001, Ruckstuhl & Neuhaus 2000).

Social, tent-building caterpillars of *Eriogaster lanestris* develop quite uniformly and thus usually build up very homogeneous groups. Individuals exhibit strongly synchronized resting phases and feeding bouts (Ruf et al. 2001b, Ruf & Fiedler 2002a) but mechanisms underlying this synchronicity are not understood at all. Synchronized activity patterns are widespread among central-place foraging lepidopteran species and are well known from other Lasiocampidae (Fitzgerald et al. 1988, Fitzgerald & Underwood 1998a), processionary caterpillars (Notodontidae, Thaumetopoeinae, Schmidt et al. 1990, Floater 1996) and the highly social Pierid *Eucheira socialis* (Fitzgerald & Underwood 1998b). However, other central place foraging species do not or only partially synchronize activities, indicating that synchronicity is no general character of tent-building caterpillars (cf. Chapter 11).

Preliminary observations on *E. lanestris* caterpillars showed that between foraging periods small groups of larvae may become active temporarily at irregular intervals. Sometimes other colony members may become activated, too, through these activity pulses, but eventually all caterpillars resume resting again (Ruf 1999). However, at the onset of a foraging bout within few minutes

the majority of caterpillars can be involved and almost all caterpillars leave the tent in a tightly synchronized manner (Balfour-Browne 1933).

Temporal synchronicity requires communication among group members. There have as yet been no studies to determine the mechanistic basis of this communication in social caterpillars. The rather well known trail pheromones (see Chapters 7+8) apparently do not play a major role in this respect. At least, no activity-stimulating potential of artificial or natural trail pheromone deposits have ever been observed so far in the Lepidoptera. It was hypothesized that synchronization could be achieved by an increasing level of restlessness among the hungry caterpillars associated with tactile cues (Fitzgerald & Costa 1999). However, movements of caterpillars cannot be monitored by direct observation because they mostly occur inside the densely woven silken tent. The use of endoscope cameras is also excluded by the multi-layer construction of the tent which would not allow for an overall view of all individuals to record their activity. Furthermore, caterpillars rapidly spin silk over any material introduced in their tent. Thus, I used an indirect technique to quantify levels of activity of the caterpillars in and on the tent by measuring vibrations of the tent surface.

This study deals with two different aspects of behavioral synchronicity in *Eriogaster lanestris*:

1. Analysis of possible mechanisms of synchronization. How do caterpillars synchronize their activity prior to the foraging bout? I specifically test the hypothesis that vibrations are a possible cue for communication in this context.
2. Experimental analysis of the impact of synchronicity. Are larger colonies better capable of synchronizing their activities? Are there any advantages of more synchronized foraging? I therefore compare five pairs of differently sized colonies with regard to their level of synchronicity during their foraging bouts.

Material and Methods

Vibration experiment

General information on vibration measurements: For measuring the vibrations of the tent I used laser Doppler vibrometry (LDV) which is a non-contact vibration measurement technique. LDV is based on the detection of the Doppler shift of coherent laser light that is scattered from a small area of the test object. The object scatters or reflects light from the laser beam and the Doppler frequency shift is used to calculate the component of velocity which lies along the axis of the laser beam. For this purpose a laser beam is divided at a beam splitter into a measurement beam and a reference beam which propagates in the arms of an interferometer. For detailed information see Polytec (2002).

Type and settings of the laser vibrometer: The instrument used was a compact laser vibrometer, type CLV 1000 (Polytec, Waldbronn). This vibrometer is an eye-safe class 2 laser instrument using a low power Helium-Neon laser. This laser produces a visible red laser beam (λ=0.6238μm) that can be focused over several meters.

Further data acquisition equipment: The laser vibrometer was connected to a data acquisition card (type PCI-Base50 provided with a 12bit A/D-module, type MAD12, BMC-Messsysteme, Maisach) placed into a personal computer (Pentium I, 133MHz). Data were sampled continuously over 24 hour intervals at a rate of 100Hz with a specialized software (NextView/NT, Ver. 3.0, BMC-Messsysteme, Maisach). In order to achieve small files, it was necessary to subdivide data in three hour intervals (containing 1,080,000 readings at a size of the file of 34.3 MB). On a second computer activity patterns were measured continuously with the help of infrared light barriers (for details see Ruf et al. 2001b, Ruf & Fiedler 2002a). It was carefully checked daily whether the internal clocks of the two computers worked perfectly in parallel.

Colonies: Due to the considerable technical expenditure only two colonies could be monitored in succession within one month. Both colonies were large in size (about 250 individuals) and were monitored during their fourth instar. Colonies were allowed to build a tent on a wooden, 3-dimensional cross (cf. Ruf & Fiedler

2002a). In order to minimize vibrations caused by movements of the building colonies were placed in an air-conditioned room ($T_{ambient}$=25°C, L:D 14:10h) in the cellar of the university building. Vibrations of the tent were measured by focusing the laser beam of the vibrometer on a small flag-like construction which was set loosely on the tent's surface and which was attached to the tent by the caterpillars by spinning silk on and around the flags some days before the start of the experiments. 'Flags' were constructed with a small piece (ca. 2x2cm) of a special coated foil (provided by Polytec, Waldbronn) that optimizes reflection. The foil was attached to an insect pin which in turn was soldered in a right angle to the crossing point of two additional insect pins that were bent at their ends to anchor them in the silk mats (Fig. 1).

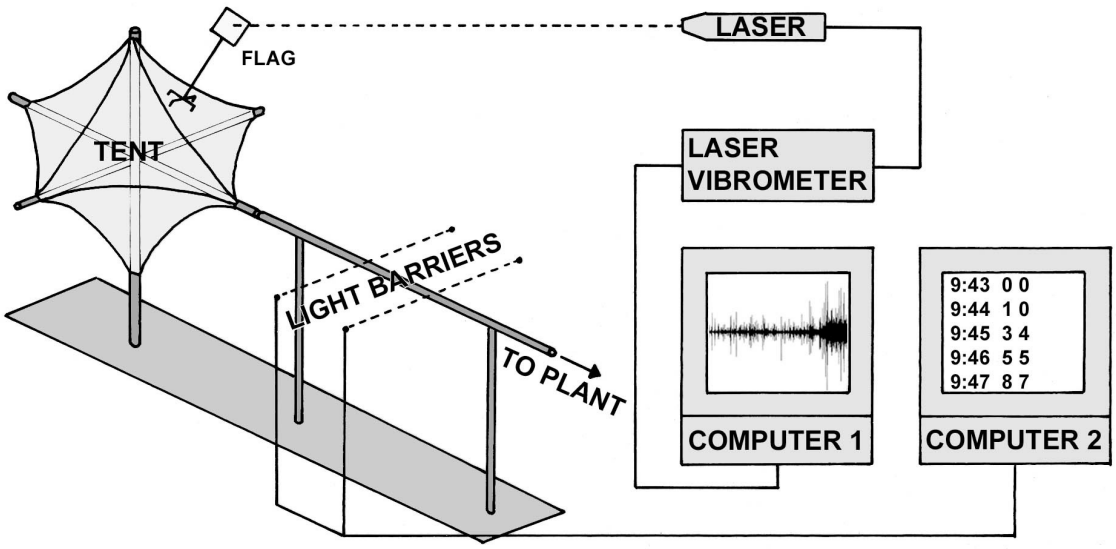

Fig. 1 – Schematized drawing of the experimental design used to simultaneously record vibrations of the tent and foraging activities of the caterpillars. Flag is not drawn true to scale but larger for better visibility.

Caterpillars continuously had access to a large bunch of their major host-plant (blackthorn, *Prunus spinosa*) over a 30cm long wooden bridge. The bunch was replaced every day. Two light barriers were attached besides the bridge for double safeguarding of the measurements of activity patterns.

Analysis of raw data: Since general programs for processing tables are not able to work with several millions of readings I used the software FlexPro 5.0 (Weisang & Co.) for analysis. In a first step data were reduced to one

measurement per minute. For this purpose I summed up the readings of one minute by calculating the integral of the area (6000 measurements each, see algorithm in Box 1).

```
SigY = '2 - Analog In.Y'
WindowSize = 6000
Count = NumberOfValues (SigY) / WindowSize
if NumberOfValues (SigY) % WindowSize > 0 Then
                Result = 0. # (Count + 1)
        else
                Result = 0. # Count
end
for i = 0 to Count-1 Do
        Ausschnitt = Trigger (SigY[i * WindowSize, (i+1) * WindowSize - 1], 0, 0,
EVENT_EXTRACT)
        Result [i] = Sum (Ausschnitt)
end
if NumberOfValues (SigY) % WindowSize > 0 Then
        Result[Count] = Sum(Trigger(SigY[Count * WindowSize, NumberOfValues
(SigY) -1], 0, 0, EVENT_EXTRACT))
end
Result
```

Box 1 – Program algorithm for reducing data using FlexPro 5.0. '2-Analog In' is the channel of the AD-DA card which was connected to the vibrometer.

Afterwards I produced combined files with vibration and foraging pattern data for further analyses. In all, 26 foraging bouts were chosen for analysis. Many other foraging bouts had to be excluded from the analysis because the spinning behavior of the caterpillars often led to a minimal moving of the flags so that the laser was not focused perfectly any more. This caused only weak signals by the vibrometer that were not suitable for further analyses. Furthermore only highly synchronized foraging bouts (i.e. those between molting phases) could be used. Files started 90 minutes before the beginning of a foraging period and ended 30 minutes after the beginning. During this time the vast majority of the caterpillars had always left the tent so that the maximum vibrations associated with each foraging bout could be determined with certainty. Since absolute measurement values for minimum and maximum vibrations highly depend on the fine tuned focusing of the laser beam, it proved to be necessary to standardize vibration recordings. For this purpose, minimum values of each set of data were set to zero and maximum values of each foraging bouts were then set as 100%. In

another analysis vibration values for the time from -90 to -30 minutes before the beginning of a foraging period were averaged and set to zero. Thus 'resting values' (see below) are either positive (first method) or oscillate around zero (second method).

Possible triggers for the caterpillars' departure: To delimit possible triggers for the synchronized departure of the caterpillars from the tent, I firstly determined some characteristic parameters from the course of the vibration recordings that could be of potential relevance for the caterpillars' decision to leave the tent. Secondly, I compared these parameter values with the time when caterpillars actually left the tent. Two different hypotheses were tested.

1. Activity could be triggered when tent vibrations exceed the resting value (background noise), i.e. there is a threshold value above which the synchronized departure of the individuals takes place. In order to determine a 'resting value' $\bar{r}$ of vibrations (i.e. a mean value for the background vibrations) vibration values were averaged from -90 to -60 minutes before the start of each foraging period (i.e. at a time during the digestion phase where any measurable vibrations were clearly not involved in foraging activities). To avoid overestimating the influence of single strong vibrations which can be either produced by individual caterpillars occasionally passing directly through the laser beam or by strong vibrations in the nearby rooms, data were smoothed using floating averages of five values each. Significant excess of the vibrations over $\bar{r}$ was scored at the time when tent vibrations exceeded the 95% confidence interval of $\bar{r}$ (i.e. when $\bar{r} \geq 1.96$ SE+$\bar{r}$). Moreover it was determined how strong vibrations were at the point of the departure of the caterpillars (percentage of maximum value).

2. The trigger could be connected with the maximal increase of vibrations, i.e. there is a temporal component of the tent vibrations that serves as a signal. To test this hypothesis, a sigmoid curve was fitted to the data (software: Datafit Ver. 8.0.32, Oakdale Engineering). I used a general model for logistic processes (Hadeler 1974, Box 2). Two points in time (x_1, x_2) are of potential biological significance here. First, the moment x_1 when there is the largest

increase in overall tent vibrations (i.e. when restlessness and thus motivation to leave is maximal among tentmates) could be related to the eventual start of a foraging period. To identify this point, I calculated the point of inflection of the sigmoid curve from the parameter estimates. The point of inflection is given by $f''(x_1)=0$ which indicates a maximum value for the first derivative, $f'(x)$. Second, the moment x_2 could be of interest, when there is the largest change in the increase rate of tent vibrations. This point is given when the second derivative, $f''(x)$, has a 'local maximum', i.e. if $f'''(x_2)=0$.

$f(x)=\frac{m}{1+a\cdot e^{-b\cdot m\cdot x}}$ where m is the maximum asymptotic value for $x\rightarrow\infty$.

$$f'(x)=\frac{a\cdot b\cdot m^2\cdot e^{-bmx}}{(1+a\cdot e^{-bmx})^2}$$

$$f''(x)=\frac{a\cdot b^2\cdot m^3\cdot e^{-bmx}\cdot(a\cdot e^{-bmx}-1)}{(1+a\cdot e^{-bmx})^3}$$

$$f'''(x)=\frac{a\cdot b^3\cdot m^4\cdot e^{-bmx}(3a\cdot e^{-bmx}\cdot(a\cdot e^{-bmx}-1)-(1+a\cdot e^{-bmx})\cdot(2a\cdot e^{-bmx}+1))}{(1+a\cdot e^{-b\cdot m\cdot x})^4}$$

Point of inflection: $f''(x_1)=0$, thus $x_1=\frac{\ln\frac{1}{a}}{-b\cdot m}$

Point of maximal curvature: $f'''(x_2)=0$, thus $x_2=\frac{\ln\frac{3+\sqrt{10}}{a}}{-b\cdot m}$

Box 2 – Mathematic equation used for regression analysis and its derivatives as well as equations for calculating local maxima.

Remarks on statistical problems: Strictly speaking, vibration data as recorded here do not represent independent data points but are temporally connected (time series). Furthermore, due to technical constraints data were taken from only two colonies and data sets can thus be seen as pseudoreplicates. However, it is most unlikely that events during one foraging period in this enemy

free situation with constant environmental conditions should influence the behavior of the caterpillars during the next foraging period. Thus, data sets were treated like independent measurements.

Synchronicity experiment

Caterpillar colonies: In order to test whether colonies of different size exhibit different synchronicity while foraging five pairs of colonies which hatched on the same day were held in an environmental cabinet. Caterpillar colonies were either obtained by breeding (second laboratory bred generation, eight colonies) or by dividing one field colony (see below). Eight colonies were assessed as being “large” or “small” since they hatched from differently sized egg clusters. “Large” colonies contained about 200 caterpillars, “small” colonies about 50 caterpillars each (all numbers ±10%). In one case, a colony collected in the field was subdivided into one large (200 caterpillars) and one small colony (20 caterpillars), the latter being established on an abandoned tent of *Malacosoma neustria* which was accepted readily as a new home base. Rearing conditions differed between experimental replicates but were identical for colony pairs (see Table 1 for details).

Activity patterns of all colonies were long term monitored with light barriers (cf. Ruf et al. 2001b). Synchronicity was assessed by calculating an index SI which is based on the density of all light barrier signals set off by the caterpillars during the course of a day (SI = Sum of signals of a day / Sum of minutes with at least one signal, cf. Ruf et al. 2001b).

For calculations of SI only days between the molting to the fourth instar and the molting to the fifth instar were taken into account which represent the synchronized phases of the fourth larval instar. Whenever caterpillars in a colony are about to molt, behavioral synchronicity declines sharply as does overall foraging activity (cf. Ruf et al. 2001b).

Statistical analysis: Collecting multiple data (i.e. indices of synchronicity) in one system (one colony) violates the postulate of independent samples. To avoid inflation of the degrees of freedom I used an ANOVA design for repeated measurements. This means that measurements of one colony are used as

multiple measurements within one system in the analysis. Because of inhomogeneity of variances data were transformed (i.e. log(sqrt(x)).

Results

Vibration experiment

Suitability of method

Figure 2 (upper graph) shows original data of the laser vibrometer of one 3h file. Although it is obvious that there is a strong increase of vibrations during the foraging phase (lower graph) differences appear rather small. However, these small differences are partly caused by the fact that it is impossible to visualize small values if more than 1 million readings are shown in one graph. Thus, single large signals within the resting phases optically lead to an overestimation of vibrations there. Figure 2 (middle graph) shows that there is actually at least a four to five fold increase of overall vibration during the foraging bouts, i.e. when the vast majority of caterpillars is active and moves over the tent. If the laser was focused perfectly even 10-fold differences between resting values and maximum vibrations during the foraging bouts occurred. During the resting phases, there was still considerable vibration (‘background noise’), that was predominantly caused by the caterpillars since there were always some single individuals that made little movements and thus caused small vibrations. In addition, vibrations of the building cannot be excluded but are considered insignificant in comparison to the large vibrations during the group movements of the caterpillars. Overall, the strength of the background noise and the absolute size of vibration scores depended on the accuracy of focusing the laser beam.

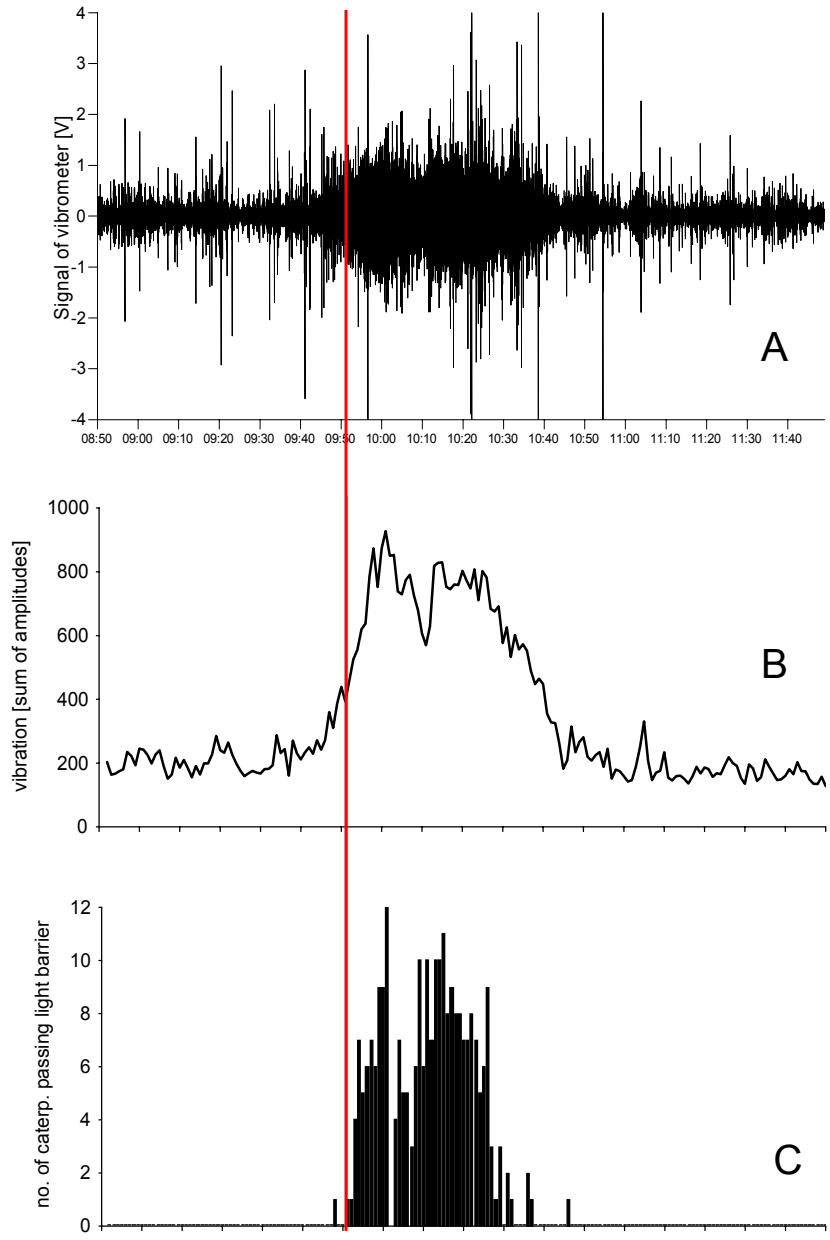

Fig. 2 – Exemplary graphs of a 3-h interval (one file). Red line indicates the start of the synchronized foraging period. **A** Original data gained from the vibrometer. The graph represents 1,080,000 data points. **B** Reduced data. (Positive) amplitudes summed up per minute. **C** Activity patterns measured with the light barriers.

Dynamics of starting phase

Although vibrations fluctuated during the course of the resting phases there was neither a regular appearance nor an increasing density of pre-foraging vibration peaks (Fig. 3). During the resting phases vibrations frequently exceeded the resting value $\bar{r}$ significantly but no caterpillar left the tent. Instead, vibrations declined again to the resting level. The mean maximum relative strength of vibrations during these pre-foraging phases reached 7.3±4.0% (min=0.9%, max=20.0%) of the absolute maximum value achieved during the foraging period later on (see below).

The phase prior to the actual beginning of a foraging period, when vibrations continuously significantly exceeded the resting value, lasted on average 19 minutes (mean±SD=18.8±6.1, min=7, max=33, N=26). In no case the departure of the caterpillars started before the resting value had been surpassed significantly for at least seven minutes.

At the point when the first caterpillars left the tent vibrations were not yet at their maximum, which means that not all caterpillars were yet mobilized. Complete mobilization took place within approximately 30 minutes after the departure of the first caterpillars. Strength of vibrations at departure varied enormously and ranged from 12.9% to 63.9% of the maximum value (mean±SD=34.2±14.2%). Thus, there was no defined strength of vibrations that could be associated with the departure of the caterpillars. However, vibrations were significantly stronger at the time of departure than during any of the pre-foraging peaks (Wilcoxon matched pairs signed rank test: $Z=4.46$, $p<0.0001$). Actually, pre-foraging peak vibrations reached only a quarter of the strength of those at the time when caterpillars left the tent.

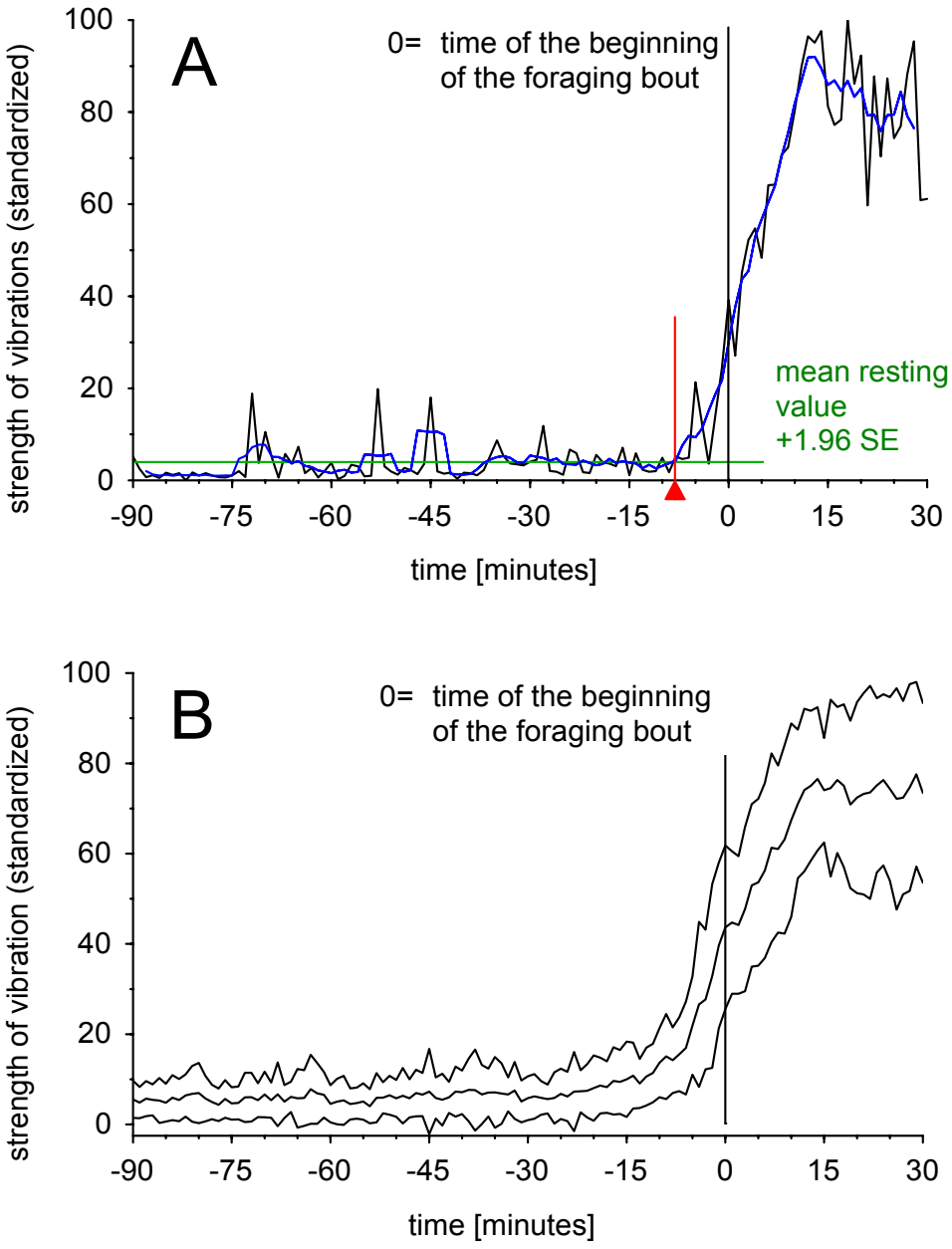

Fig. 3 – Dynamics of vibrations 90 minutes before to 30 minutes after the beginning of a synchronized foraging bout (x=0). Vibrations were standardized (the minimum is set to zero, maximum = 100). **A** Exemplary graph. Black line: Original data, blue line: smoothed data, red arrow: time, when tent vibrations exceed the resting value significantly for the last time before the departure of the caterpillars. **B** Trajectories of mean±1SD of all 26 data sets available.

The general logistic model fitted almost perfectly to the data (Fig. 4). If data of 26 starting phases were pooled, the point of largest change in the increase rate (x_2) was eight minutes before the departure of the caterpillars (x_2=-8.21, 95% confidence interval: x_2=-9.18 – -7.38). Regression analyses for the 26 individual data sets were more scattered but revealed a similar result (mean x_2(N=26)= -6.35±2.90).

The point of inflection (i.e. the time when the increase of vibrations was maximal) calculated from the parameter estimates is practically identical with the time when caterpillars start leaving the tent (x_1=0.37, 95% confidence interval: x_1=0.07–0.61). Thus, the caterpillars' departure precisely coincided with the time when the increase of the vibrations from one minute to the next was maximal. Again, regression analyses for the 26 individual data sets were more scattered but revealed a similar result (mean x_1(N=26)=0.98±3.96).

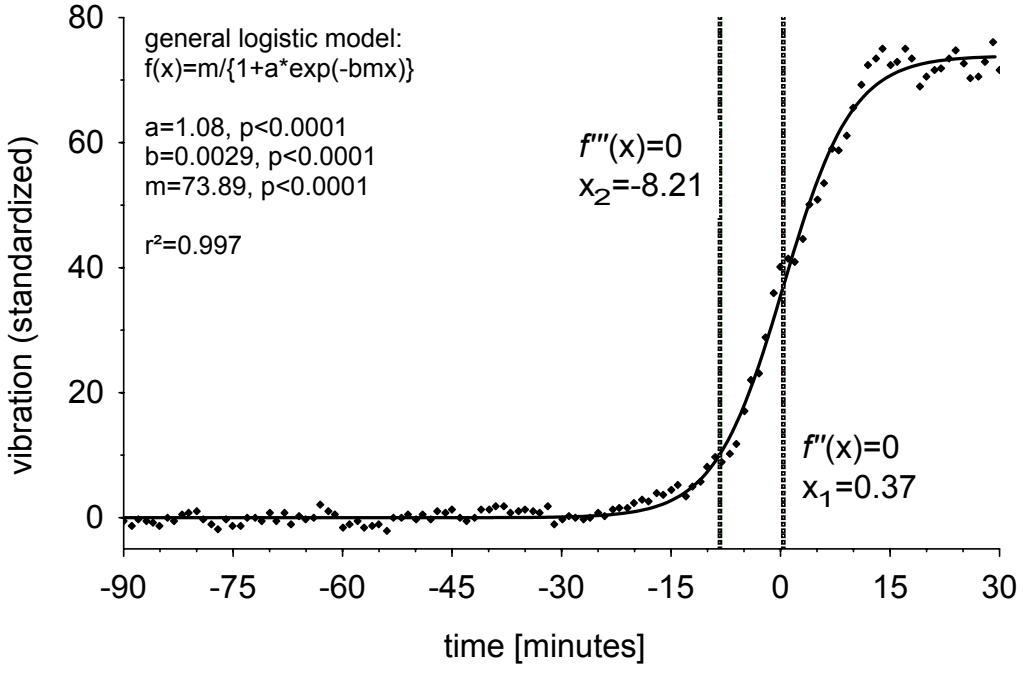

Fig. 4 – Logistic function modeling the dynamics of the phase 90 minutes before to 30 minutes after the beginning of a foraging period (x=0). Data are average values of 26 sets of data. The point of inflection of the curve is nearly identical with the time when caterpillars start leaving the tent.

Synchronicity experiment

Small colonies showed a remarkable lag of development compared to larger colonies (Table 1). Even the development of colony V/2, which was only subdivided before molting to the fourth instar was retarded for 1.5 days when molting to the fifth instar.

Table 1. Rearing conditions, size, hatching and molting times for the five pairs of colonies of *E. lanestris* used for this study.

Colony	Rearing conditions	Size	Hatching date	Molting to 4^{th} instar	difference
I/1	22 °C / 15 °C	large	24/04	13/05	4 days
I/2		small		17/05	
II/1	22 °C / 15 °C	large	28/04	18/05	4 days
II/2		small		22/05	
III/1	20 °C const.	large	02/05	23/05	2 days
III/2		small		21/05	
IV/1	20 °C const.	large	03/05	16/05	2 days
IV/2		small		18/05	
V/1	22 °C / 15 °C	large	unknown	identical	-
V/2		very small			

To understand influencing factors on synchronicity I first analyzed whether rearing conditions (i.e. temperature regime in the environmental cabinet) influenced synchronicity of caterpillars by a multifactor ANOVA design. Rearing conditions had no influence on synchronicity ($F(2, 1)=0.77$; $p=0.52$) and was therefore subsequently excluded from the analysis. Group size and 'within-groups' (i.e. temporal variability between the repeated measurements within one colony) proved to be significant ($F_{size}(1, 4)=17.51$; $p=0.014$; $F_{within\text{-}groups}(6, 24)=2.83$; $p=0.032$). No interactions between the factors were found, indicating that temporal fluctuations in synchronicity existed, but did not vary consistently between group sizes.

Testing for size and within-group factors only revealed that the influence of group size was predominant ($F_{size}(1, 8)=28.75$; $p=0.0007$). Differences between

the colony pairs proved to be important as well but were far less pronounced ($F_{within\text{-}groups}$ (6, 48)=3.18; p=0.010). Again, no interactions of factors were found.

Figure 5 shows that synchronicity of behaviors was always highest in large colonies. It is also apparent that SI values were overall much more similar across large colonies than in small colonies. Thus, the significant within-group variation (see above) is mostly due to the more variable behavioral synchrony exhibited by small colonies.

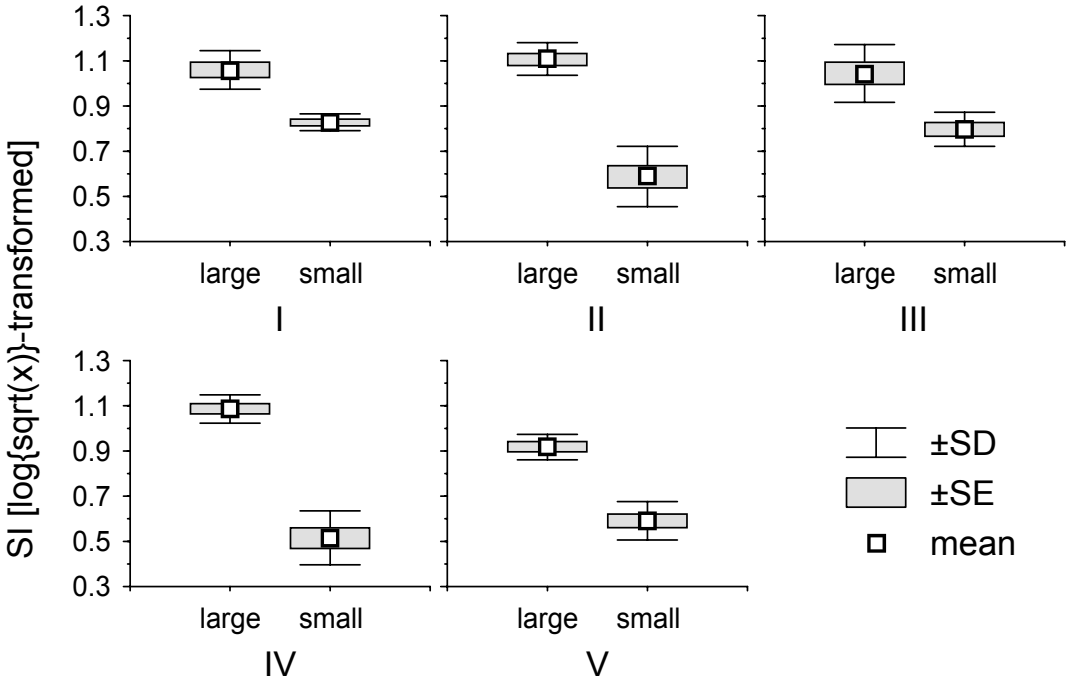

Fig. 5 – Influence of group size on synchronicity of daily foraging in colonies of *E. lanestris.* Synchronicity is assessed by using an index SI that integrates density of signals over the course of one day. Sample size: N=7 days for all colonies. Roman numbers refer to the colony numbers from Table 1. Size of the index was significantly higher for large colonies in all colony pairs.

Discussion

It is obvious that social groups can only be coherent if their members remain in the same place at the same time and, for this to occur, it may often be necessary for them to engage in the same activity all at a time (Conradt & Roper 2000). Casey et al. (1988) speculated that there are strong selective pressures for synchronous foraging in colonies of the Nearctic eastern tent caterpillar, *Malacosoma americanum*. These are assumed to having given rise

to group mediated behavioral patterns that override the tendency of individuals to follow foraging schedules dictated by their own hunger levels. It is generally acknowledged that for these tent caterpillars high synchronicity is especially helpful for thermoregulation (through collective resting and basking) or for detecting and exploiting profitable feeding sites (by collective searching and feeding). However, since social, central place foraging caterpillars use a fixed home base and trail-based orientation in the vicinity of their tent (Fitzgerald 1995, Fitzgerald & Underwood 1998a,b, Ruf et al. 2001a), it is not inevitably necessary to synchronize both, resting phases and feeding activity. For example, caterpillars of *Eriogaster catax* exhibit collective resting phases when the majority of the caterpillars of the colony lie in a tight group on the surface of the tent and bask (cf. Chapter 11). Nevertheless, simultaneously small subgroups are foraging, i.e. they are asynchronous. Thus, resting caterpillars of *E. catax* benefit from the presence of their siblings by enhanced social thermoregulation (see Joos et al. 1988, Ruf & Fiedler 2000, 2002b for details), but individuals still follow their own feeding rhythm. Furthermore, analysis of the foraging behavior of caterpillars of *Malacosoma americanum* has demonstrated that single individuals are able to find new, profitable feeding sites and recruit the whole colony to these places (Fitzgerald & Underwood 1998a). Once such pheromone paths are laid it does not seem crucial that the rest of the colony forages *en masse*. In *Malacosoma neustria*, individuals show marked asynchrony and individualism while feeding but are nevertheless able to maintain more or less closed groups during the first four larval instars (Peterson 1988, Chapter 11). Obviously, behavioral synchronicity is no necessity for social, tent-building caterpillars.

This study demonstrated that in *E. lanestris* the size of a colony significantly influenced synchronicity among individuals. Large colonies were more likely to integrate and behave as a unit and synchronicity was less variable across colonies. Furthermore, large colonies also developed faster compared to small colonies despite constant environmental conditions. Developmental differences at different group sizes are usually assigned to social facilitation in feeding (e.g.

Tsubaki 1981, Tsubaki & Shiotsu 1982, Lawrence 1990, Clark & Faeth 1997, Denno & Benrey 1997, see also Chapter 12). However, 'small' groups in my experiment still contained more caterpillars than would be physically able to feed on one single blackthorn leaf, even in the first instar. Furthermore, blackthorn leaves do not show any obvious defensive structures that need to be overcome by communal feeding. Thus, communal feeding is not expected to facilitate nutrient intake in this particular case.

In his detailed life-history documentation on *E. lanestris*, Balfour-Browne (1933) also reported that large colonies were much more successful than small ones and grew faster but he did not show any concrete data nor did he give any possible explanation. Possibly, faster development may be directly caused by a higher synchronization of individuals which means that individuals spend less time for searching food and more time for digestion (energy saving strategy). Fitzgerald and Visscher (1996) showed that isolated larvae of the eastern tent caterpillar, *Malacosoma americanum*, were more active but developed more slowly compared to grouped caterpillars. However, these effects already stabilized in groups of few (five to ten) individuals while my 'small' groups contained 20-50 individuals and were able to build normal although small tents. It is therefore conceivable that developmental differences are also influenced by thermoregulatory differences between groups of different size. Although caterpillars in the environmental cabinets were not able to use solar radiation for basking, metabolic heat production could be responsible for the observed effects (Ruf & Fiedler 2000). However, it is presently unknown how net profits of metabolic heat production depend on group size.

Synchronization requires communication among the members of a group. Otherwise, tightly synchronized, fast departures of the caterpillars at the beginning of a foraging period are not imaginable. Balfour-Browne (1933) also reported that the majority of the colony 'suddenly' became active and moved off to feed without any obvious trigger.

This study shows that there is no continual building up of activity peaks of increasing strength. Instead, resting values are frequently exceeded but no

foraging period follows such peaks. However, before the first caterpillar leaves the tent, vibrations significantly exceed the level of any of the pre-foraging activity bursts. Actually, this phase proved to be longer than was assessed from observations merely recognizing movements on the tent and nearly lasted 20 minutes. Nevertheless, there was neither a defined threshold value, the excess of which served as a signal for the synchronized departure, nor was it necessary to activate all tentmates to start a foraging bout. Furthermore, the time x_2, when the 'restlessness' among caterpillars built up fastest did not serve as a trigger for the departure as well, since it occurred more than eight minutes prior to the start of the foraging bout. After the exclusion of many possibilities it seems most likely that the time x_1, when the increase of vibrations and therefore the mobilization rate of tentmates was maximal and which clearly coincided with the starting time of the foraging period, may serve as trigger for the synchronized departure of the caterpillars. I therefore hypothesize that substrate-borne vibrations are used by the caterpillars to sense the readiness of their tentmates to leave the tent. Of course, it cannot be excluded from my experiments that chemical cues might also help to synchronize activity. However, some considerations make chemical signaling unlikely in this context. For example, if caterpillars rest on the tent before departure, air movements should quickly disperse any volatile pheromone. Otherwise, the pheromone would be restricted to some regions of the tent due to its multilayer structure if caterpillars rest in the tent. Moreover, caterpillars with their small antennae have a lower morphological capacity to receive volatile signals. In contrast, substrate-borne vibrations can easily be perceived at any region of the tent and therefore could serve as an efficient one for each caterpillar to sense the overall 'willingness' of tentmates to commence foraging.

In the last three decades it has become clear that the use of vibrations in animal communication is much more widespread than previously thought (Hill 2001). Vibration may provide information used in predator-prey or mutualistic interactions, recruitment to food, mate choice, intrasexual competition and maternal/brood social interactions in adult insects (e.g. McVean & Field 1996, Roces & Hölldobler 1996, Hill & Shadley 2001), Lepidopteran pupae and larvae

(e.g. DeVries 1990, Bacher et al. 1997), spiders (e.g. Fernandez Montraveta & Schmitt 1994, Schüch & Barth 1990) and other arthropods (e.g. Aicher & Tautz 1990) or even elephants (e.g. O'Connell et al. 1997). However, most papers studying vibrations as a means of communication concentrated on discrete signals produced during special (often ritualized) behavioral sequences, where the signals usually show strong regularity.

In tent living caterpillars vibrations are a by-product of locomotion that could secondarily be used for communicative purposes. As a consequence, vibrations are highly irregular and it is therefore their timing and relative increase in intensity which could carry the relevant information. Since tents are a highly inhomogeneous substrate (which has many consequences for the transmission and frequency characteristics of substrate-borne vibrations) it seems quite unlikely that there is more information encoded than just the level of restlessness among tentmates.

If *E. lanestris* larvae really use vibrations as cues, they must be able to sense these. The perception of air-borne vibrations by specialized receptor hairs has been documented for a number of butterfly and moth caterpillars (Tautz 1989) and has been studied in much detail for caterpillars of the cabbage moth (*Mamestra brassicae*: Tautz 1977, 1978, Tautz & Markl 1978). Gregarious sawfly larvae that are morphologically and ecologically most similar to social lepidopteran caterpillars have been shown to use substrate stridulation for communication (Hograefe 1984). In the sawfly *Hemichroa crocea* foraging larvae regularly scratch over the leaf surface by stretching out and curling their abdomina. Scratching frequency is thought to indicate patch quality and shall keep the colony together. Although the sensory basis of receiving these signals was not finally clarified sensilla-like structures were supposed to play a role. However, the perception of strong substrate vibrations like those of the tent need no specialized hairs but can also be perceived by chordotonal organs that occur throughout the peripheral regions of the body and in the legs (so-called subgenual organs) of insects (Chapman 1982, Dettner & Peters 1999).

To conclude, this study has accumulated the first (although still indirect) evidence that *Eriogaster lanestris* may use tent vibrations to synchronize activity at the beginning of a foraging bout. This mode would be a most efficient and most economical one. The departure of the caterpillars could be triggered by a significant increase in the level of vibrations of the tent and precisely coincides with the moment when the rate of mobilizing tentmates is maximal. The emergent behavioral synchronicity turned out to depend on groups size and conferred developmental advantages to the caterpillars in an enemy-free situation. These advantages are likely to be even larger when sun-basking or enemy avoidance are considered, as they occur in the field.

Acknowledgements

I am very grateful to Bernd Kornmaier for technical advice and help during the evaluation of 6.6 gigabytes of data. I also thank Jörg Hager for maintaining colonies and a short mathematical lesson closing gaps in memory. Financing of the rent for the laser vibrometer by the Deutsche Forschungsgemeinschaft (Fi 547/8-1) is kindly acknowledged.

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Chapter 10

Colony survivorship of social caterpillars in the field: A case study

Submitted to a peer reviewed journal as: Ruf, C.; Fiedler, K.: Colony survivorship of social caterpillars in the field: A case study of the small eggar moth (Lep., Lasiocampidae).

Abstract:

This study investigates temporal survivorship and its spatial variation of 100 caterpillar colonies of the small eggar moth, *Eriogaster lanestris*. The field study took place at four sites in Northern Bavaria in May and June 2002. Egg clusters were obtained from females mated in the laboratory and were transplanted into the field just before hatching of the young caterpillars. Colony mortality rate was rather constant over time (total: 48%) and not mainly restricted to the earliest instars. There was no spatial heterogeneity in mortality. The inability to build an initial tent or the later loss of the tent accounted for 71% of total colony losses. Strong rainfalls had a severe influence on the constitution of tents. The impact of climatic factors and predation on colony survival as well as the importance of the tent structure for the survival of social caterpillars are discussed.

Introduction

Lepidopteran larvae are most vulnerable in the earliest stages of their lives. Since natural enemies are supposed to be the dominant cause of mortality (Cornell et al. 1998) most caterpillars rely on a hidden life habit with cryptic coloration and show behavioral adaptations that reduce their risk to be detected by visually oriented natural enemies (e.g. commuting between feeding areas and hidden resting areas, nocturnal activity, Heinrich 1979, 1993, Stamp & Wilkens 1993).

Only few lepidopteran caterpillars (<3% of the species worldwide, Costa & Pierce 1997) live gregariously or ‘socially’, sometimes in groups of several

hundreds of individuals. Although gregarious caterpillars may benefit from living in groups in several ways, for example by maximizing growth through efficient thermoregulation (Joos et al. 1988, Ruf & Fiedler 2000, in press) or by social feeding facilitation (Clark & Faeth 1997, Denno & Benrey 1997), they are very conspicuous at the same time (for a general overview see: Fitzgerald 1995). Thus, groups may be at a high risk to become totally extinct once they have been detected by visually hunting predators like birds or by predators that recruit further nestmates to food (e.g. ants, wasps).

Many gregarious species show a warning coloration (Sillen-Tullberg 1988, Vulinec 1990) and are supposed to be chemically or structurally defended, which enables these caterpillars to behave conspicuously (Heinrich 1979, 1993, but see also Ruf et al. 2001b). Several studies showed that the effect of aposematism may be enhanced by gregarious life habits because grouping makes the aposematic signal more effective by generating a greater aversion in predators (e.g. Gamberale & Tullberg 1996, 1998). Nevertheless, unless unpalatability is tested explicitly for any species this attribution always remains doubtful. In addition, the effectiveness of any defensive structures may vary according to the larval stage as well as between various types of predators (den Boer 1971).

We here use the small eggar moth, *Eriogaster lanestris*, to address the trade-off of social behavior, and the concomitant association with a communal silken tent, with larval survivorship under the influence of natural predation and adverse weather conditions.

Caterpillars of *E. lanestris* hatch in spring few days after budbreak and live together in and on a silken tent until the very end of their development, when they finally leave the tent and pupate some meters away (Balfour-Browne 1933). Females lay all their eggs in one single cluster and all siblings stay together for their whole development. Caterpillars start building the tent right after hatching, and expand the structure throughout development. Tents may achieve an estimated volume of 750cm³ and are visible over a distance of tens of meters by a human observer. Since the tent does not include any resources, caterpillars must leave the tent for every food intake (i.e. they are central-place

foragers according to Fitzgerald & Peterson 1988). Individuals are highly site tenacious and always return to the tent after feeding even if the tent has been severely destroyed. This feature is advantageous for survival analyses since re-locating colonies over a longer period of time is possible. Young caterpillars (L1-L3) are totally black (to the human observer) and covered with white setae, whereas fourth instar caterpillars develop additional tufts of shorter red urticating hairs, which are even more pronounced in the fifth (last) instar.

Many aspects concerning the advantages of living in a group and mechanisms underlying group behavior in *E. lanestris* have been analyzed in recent years (Ruf & Fiedler 2000, 2002a,b, Ruf et al. 2001a). Data on the survival of the caterpillars in the field are crucial for understanding the costs associated with sociality in this species, since the destruction of a colony means the total loss of a female's offspring. It is the purpose of this study to investigate temporal survivorship and its spatial variation in *Eriogaster lanestris* on the colony level under field conditions.

We expect two contradictory scenarios: If defensive features of the caterpillars are essential, we expect high initial mortality in the earlier instars (L1-L3) and a clear reduction of mortality after the molt to the fourth instar when urticating hairs have developed. Alternatively, mortality should influence all instars in the same manner, if predation by different predators feeding on the different larval stages is equally significant. Moreover, climatic factors are expected to influence especially the younger caterpillars that have not yet build a tent or which have only a small tent.

Material and Methods

Study sites: All study sites were situated in the region around Bayreuth (Germany, Northern Bavaria, see Figure 1 for relative geographical positions) and can be characterized as typical xerothermic habitats on limestone underground with blackthorn bushes and/or hedgerows. All sites were either southwest exposed slopes or open plateaus. At sites HP and PB the authors have found naturally occurring tents of this species several times between 1999 and 2002. The site HL had previously been only scarcely covered with

blackthorn bushes but presently blackthorn shrubs rapidly spread over the whole site. The site BB is a formerly military training area maintained through regular pasturing by sheep and goats but also experiencing massive encroachment by blackthorn. For BB the occurrence of *E. lanestris* has been reported for at least the end of the 1970s (Wolf 1982).

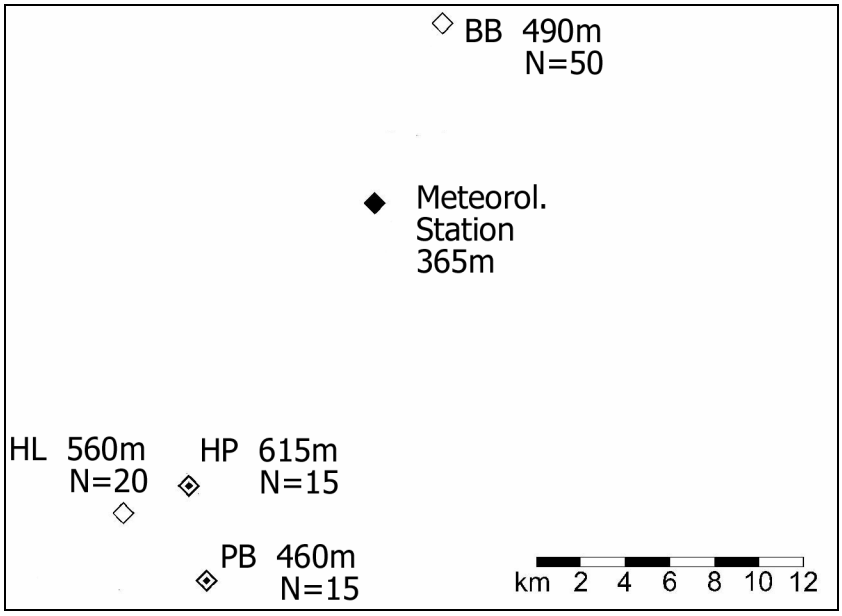

Fig. 1 – Relative geographical position of the four study sites with statements to altitude (meters above sea level) and number of experimental colonies exposed per site. Marked diamonds denote sites with documented naturally occurring populations of *E. lanestris* in 2002.

Animals: 100 egg clusters laid on small twigs were attached between 8 – 10 May with small pieces of wire to branches of blackthorn, *Prunus spinosa*, which is one of the preferred natural host-plants in Southern Germany (Ebert 1994). We preferentially chose the top of smaller bushes (height <ca. 1.7m) or the sun-exposed side of higher plants (height 2-3m). All egg clusters were placed 0.5-1.5m above ground (depending on the height of the plant) and were marked with small numbered labels for later relocation and identification. The number of egg clusters exposed per site varied (cf. Fig. 1) depending on the size of the area and the number of suitable blackthorns.

All egg clusters stemmed from a laboratory bred population (third laboratory generation). Originally, about 500 caterpillars from three tents had been collected at the site HP in 1999. For breeding we attempted to use only males

and females from different colonies. Thus, inbreeding should not have influenced our experiments since in each year subsequent generations consisted of 1000-1500 individuals descending from at least 10 different colonies.

Cocoons were over-wintered in a refrigerator at 4°C under a 6:18h (L:D) light regime. Moths hatched within a few hours after they were transferred to room temperature in April. Groups of five males and five females were put in a plastic box (ca. 13l volume, customary pet boxes) with blackthorn twigs for egg deposition. Resulting egg clusters were stored in a shaded cage outdoors to maintain them under semi-natural humidity conditions and to prevent early hatching.

Monitoring: After exposure of egg masses at the field sites hatching of the caterpillars and development was monitored once a week (Table 1).

Table 1. Exposure dates, census dates and progress of larval development for 100 experimental *Eriogaster lanestris* colonies monitored in 2002 in the region of Bayreuth.

	North (site BB)		South (sites HP, HL, PB)	
Start	8 - 9 May	exposure	10 May	exposure
Census 1	15 May	first caterpillars hatched	20 May	60 % of colonies hatched
Census 2	22 May	L1, most tents established	26 May	L1, most tents established
Census 3	28 May	L1/L2	1 June	L1/L2
Census 4	4 June	L2/L3	9 June	L2/L3
Census 5	12 June	L2-L4 (most L3)	16 June	L2-L4 (most L3)
Census 6	19 June	L3-L5 (most L4)	22 June	L3-L5 (most L4)
Census 7	26 June	most tents abandoned	29 June	L5 or abandoned

Since egg clusters were not attached to the blackthorns before budbreak to prevent caterpillars from hatching too early, the developmental status of exposed caterpillars did not perfectly match larval ontogeny of naturally occurring field colonies that proved to be one larval stage ahead (N=2 colonies, sites HP and HL). Nevertheless, some of the experimental colonies finished development before the younger one of the natural colonies. This means that

our colonies were not much out of phase and experienced predation and weather in the same way as natural colonies.

This study focuses on colony, rather than individual, survivorship since caterpillars may hide in the tent during rain or during moderate solar irradiation (Ruf & Fiedler, 2002b) and are not visible to the observer. Thus, the precise number of caterpillars cannot be specified and even the larval stage of the majority of the colony members may not be definable at each census.

The condition of the colonies was evaluated by using the descriptors listed in Table 2. If possible, the cause of the damage of a tent was recorded as well. While predation by invertebrates does not leave obvious marks on the tent, predation by birds is easily visible by the large holes in the tent occurring in the middle of the silk mats. In contrast, damage caused by rain is clearly distinguishable from that by birds since ruptures due to rain arise from the edges of the mats and stretch in the direction of the silk filaments.

Table 2. Descriptors for the condition of colonies (pertinent to either egg cluster or tent).

Descriptor	Explanation
hatched	caterpillars just hatched, no tent yet established
infertile	no caterpillars hatched from the egg clusters within three weeks
no tent	caterpillars without tent (just after hatching, because of small number of caterpillars, or after destruction of tent)
intact	tent with no physical damage
repaired	tent was obviously damaged, but new silk has been spread over the holes
damaged	egg cluster: parts are missing; tent: shows holes, not yet repaired
destroyed	egg cluster: no eggs left; tent: completely destroyed, but caterpillars still alive
abandoned	tent abandoned, occasionally fifth (=final) instar caterpillars in the nearby surroundings
extinct	colony completely destroyed, no caterpillars nearby

Climatic conditions: Climatic data were provided by the meteorological station at the University of Bayreuth. Since all study sites are less than 20km away from the meteorological station these data should be precise enough to show the overall weather conditions during the time of the study. More precise data for the southern sites are unfortunately not available.

Results

During our observations mean daytime temperature (0700-1900h) was 18.9°C, mean nighttime temperature (2100-0500h) was 11.3°C. Maximum temperature was 33.0°C and nighttime minimum temperature was always above 0°C (minimum: 2.8°C) (cf. Fig. 2).

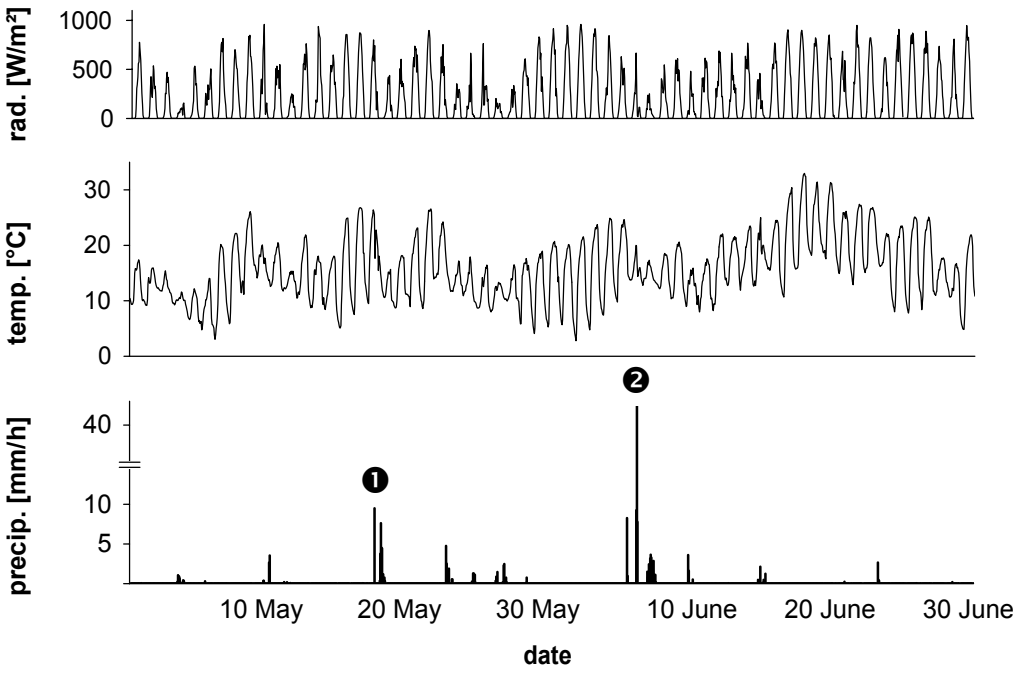

Fig. 2 – Climatic conditions (short-wave radiation, temperature, and precipitation) in Bayreuth, May and June 2002. Note the interruption of the y-axis in the undermost graph. Numbers: 1 = raining period during hatching time. 2 = heavy rainfalls during instars L2/L3.

Table 3 shows that mean temperature and the sum of precipitation were higher in May and June 2002 compared to the average of the last 30 years.

Table 3. Mean temperature and mean sum of precipitation in Bayreuth (Northern Bavaria) in May and June between 1971 and 2001 and in the year of the study.

	May 1971-2001	June 1971-2001	May 2002	June 2002
Temperature [°C]	12.0	14.9	14.1	17.5
Sum of precipitation [mm]	56.3	78.9	76.5	113.4

On June 6 unusually strong rainfall occurred with 43l/m²×h. Comparisons to other years (1992-2001) show that the monthly sum of rainfalls may vary considerably among years and has been similarly high in other years but such extreme rainfall events in May and June are exceptionally rare in this region (Fig. 3).

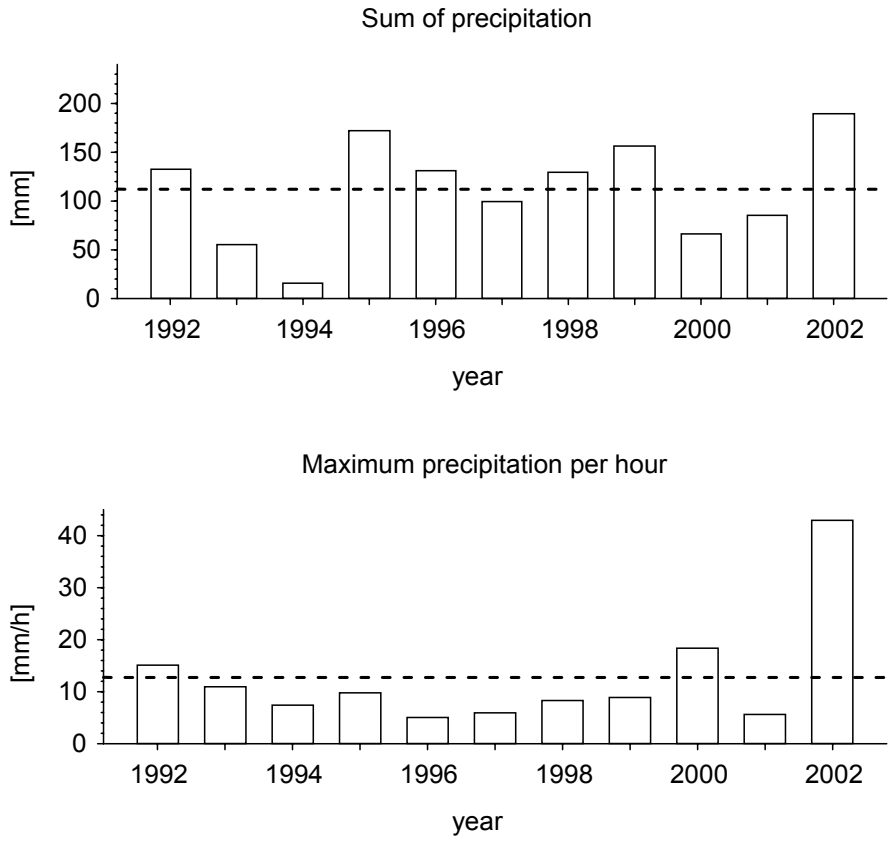

Fig. 3 – Sum of precipitation and maximum precipitation per hour in May and June from 1992 to 2002. Total precipitation in 2002 was not too different from other years but the heavy rainfalls on June 6 (60mm within three hours) are extremely unusual for the region. Dashed lines indicate mean values of the eleven years that were taken into account. Data for 1997 are incomplete and contain data of only three weeks.

Most caterpillars hatched between May 15 and May 20 (i.e. ca. one week after exposure in the field) and left their tents around the end of June (northern colonies at site BB always a few days ahead compared to the colonies at the more southern sites HL, HP, and PB). Thus, the complete development of the caterpillars lasted approximately 6-7 weeks, because caterpillars pupate very soon after leaving the tent (C. Ruf, pers. obs.).

Five of 100 egg-batches failed to eclose. Obviously, these eggs were not fertile. In two further cases the egg clusters were totally destroyed prior to eclosion. We do not know whether they were actively destroyed by a predator or if the adhesion of the eggs was solved during rain or because of the shrinkage of the dead branch, on which the egg-batch had been deposited in the laboratory.

In 94.2% of all colonies that were successful in building a tent (N=69), the tent was directly built on the egg mass. Only four colonies built their tent about 30cm above the egg mass. In most cases, the same tent was used (and expanded) over the whole development. Occasionally (N=6 colonies), caterpillars moved to another place to start tent-building for a second time. This was always a web of the ermine moth (*Yponomeuta padella* Linnaeus 1758), which was then expanded.

Overall, 52 colonies survived until census 7 when the caterpillars had reached their final instar (Table 4). Survival here means that a least one mature caterpillar could be traced which could unequivocally be attributed to a given colony. There was no significant difference in the mortality rate over time between the four study sites (Kaplan-Meier analysis, Fig. 4). Overall mortality was constant over time, i.e. there was a strong negative correlation between the number of colonies surviving and time ($r=-0.95$, $r^2=0.91$, $p<0.0001$).

Table 4. Number of colonies surviving with at least one individual until fifth instar.

Site	Number of colonies surviving until fifth instar / total number	Percentage colonies surviving
BB	22 / 50	44%
HP	10 / 15	67%
PB	9 / 15	60%
HL	11 / 20	55%
total	52 / 100	52%

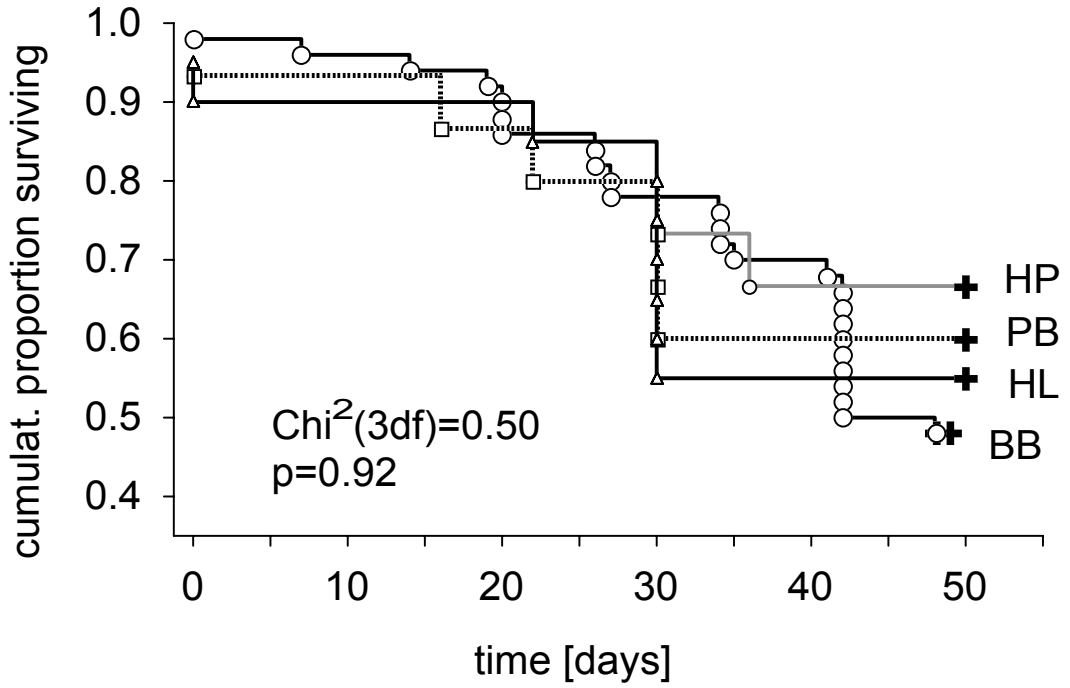

Fig. 4 – Cumulative proportion of colonies surviving at the four study sites. Statistical figures relate to Kaplan-Meier survival analysis and indicate that survival was not significantly different between the four sites.

Successful construction of a tent seems to be crucial for the survival of the caterpillars (cf. Fig. 5). Of 24 caterpillar groups that had not been successful in building an initial tent by the second census (i.e. within approximately one week after hatching) 18 became extinct which amounts to 43.9% of the total losses that occurred after hatching. From the remaining six hatchling groups only two were able to establish a tent even later during the second instar. The other four groups survived without having built a tent, but dwindled to a final group size of only one individual each until census 7. Even later in the larval life, complete destruction of the tent strongly increased the extinction risk of these colonies (Fig. 5). Only two out of 15 colonies that lost their tent (between census 3 and 7) succeeded in rebuilding a tent and two further such colonies survived without a tent, whereas the other eleven colonies died out within 1-3 weeks after tent destruction.

Thus, 70.7% of the overall colony mortality after hatching was caused by or associated with the preceding loss or non-existence of the tent. Probability of survival was significantly higher (nearly 3-fold) after the successful construction

of a tent and again significantly increased (again, nearly 3-fold) when the tent remained intact or when the caterpillars were able to repair the tent before it was totally destroyed (see statistics in Fig. 5).

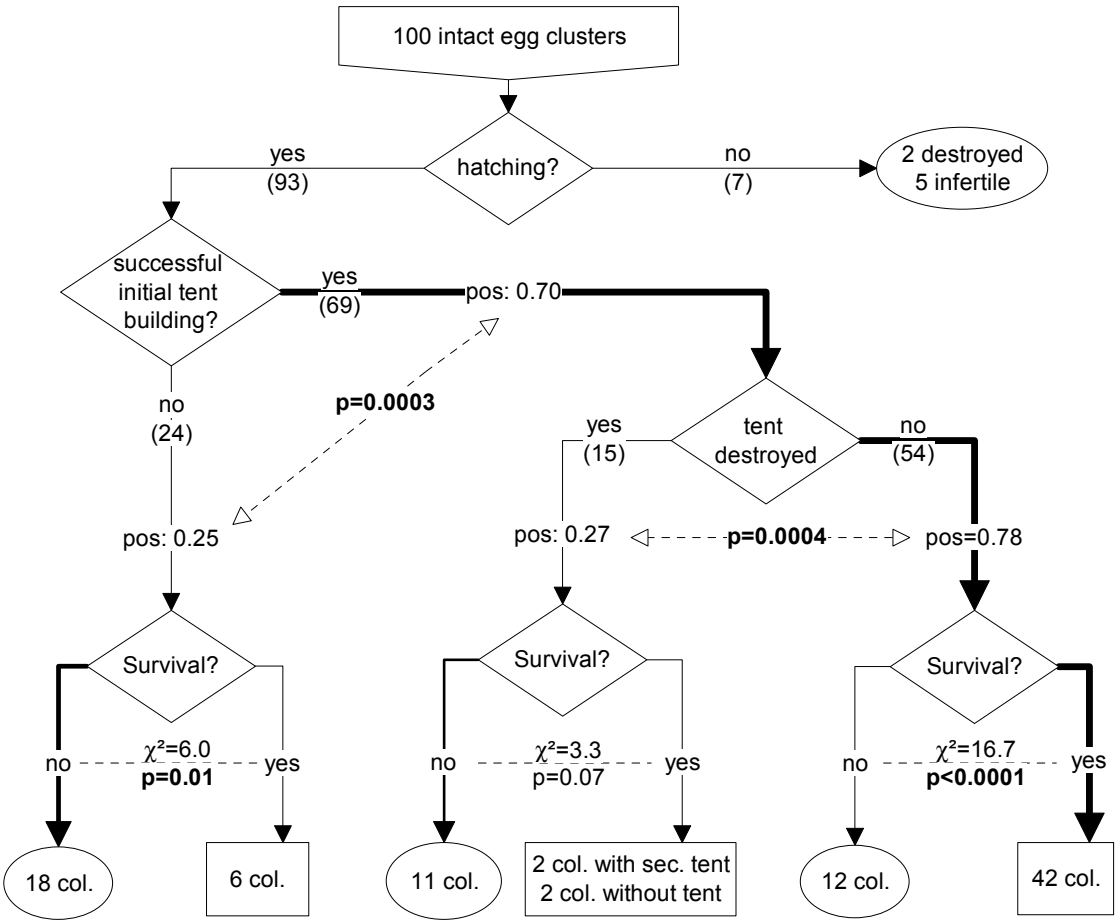

Fig. 5 – Flow-chart diagram illustrating the fate of the colonies. Numbers in parentheses = number of colonies. pos = probability of survival. Statistics: Comparison of actual survival probabilities at a given point: Fisher's exact test, test for deviation from equal distribution: Chi-square test (1df). All probability values printed in bold are significant after sequential Bonferroni correction. Different width of arrows indicates pathways with significant differences in survival.

Predation is generally supposed to be the major cause for larval mortality in Lepidopteran larvae. Although the weekly controls of the colonies provided only snapshots and observations lack completeness, predation was observed by ants (*Lasius* sp.; *Formica*. sp.), bugs (Fam. Miridae), spiders, and beetles (Fam. Cantharidae) during the earlier larval stages. Only two of 11 colonies at which predation by invertebrates was directly observed became extinct by the next

census. Again, both of these colonies had no tent for their protection. Predation by birds directly at the tents did not occur before census 5 at site HP and census 6 at site BB (i.e. when pre-final instars equipped with urticating hairs were present). None of the tents with obvious damage by birds (N=5) was completely lacking caterpillars, and in no case bird attacks led to the complete extinction of a colony.

Besides predation, climatic factors, particularly rain, had a severe impact on the condition of the colonies in our experiments. Figure 6 shows that all tents at site BB and the majority of tents at the southern sites were damaged after the severe rainfalls on June 6. Most tents showed a noticeable reduction of volume at census 5 compared to census 4 and tents were probably temporarily (i.e. until the damage was repaired and the tent was newly expanded again) not habitable for the caterpillars after the severe rainfalls.

Discussion

Contrary to our expectations, colony mortality was not restricted to, nor particularly intense, during early instars in *E. lanestris* in our study but was very constant over time. This suggests that different types of natural enemies were equally important over the whole development. In spite of the possession of urticating hairs, colony mortality did not drop distinctly in the last larval stages.

Comparable data from other social, tent building caterpillars are rare. In a field study conducted in Georgia, USA, caterpillars of *Malacosoma americanum* finished their development within 7-8 weeks (Costa 1993). Climatic conditions in the Georgia piedmont in March and April and in the region of Bayreuth in May and June are comparable with respect to mean temperature (Georgia: March: 13.7°C, April: 16.0°C), whereas precipitation is even higher in Georgia (March: 207.5mm, April: 54.6mm) (State Climate Office, University of Georgia, Athens).

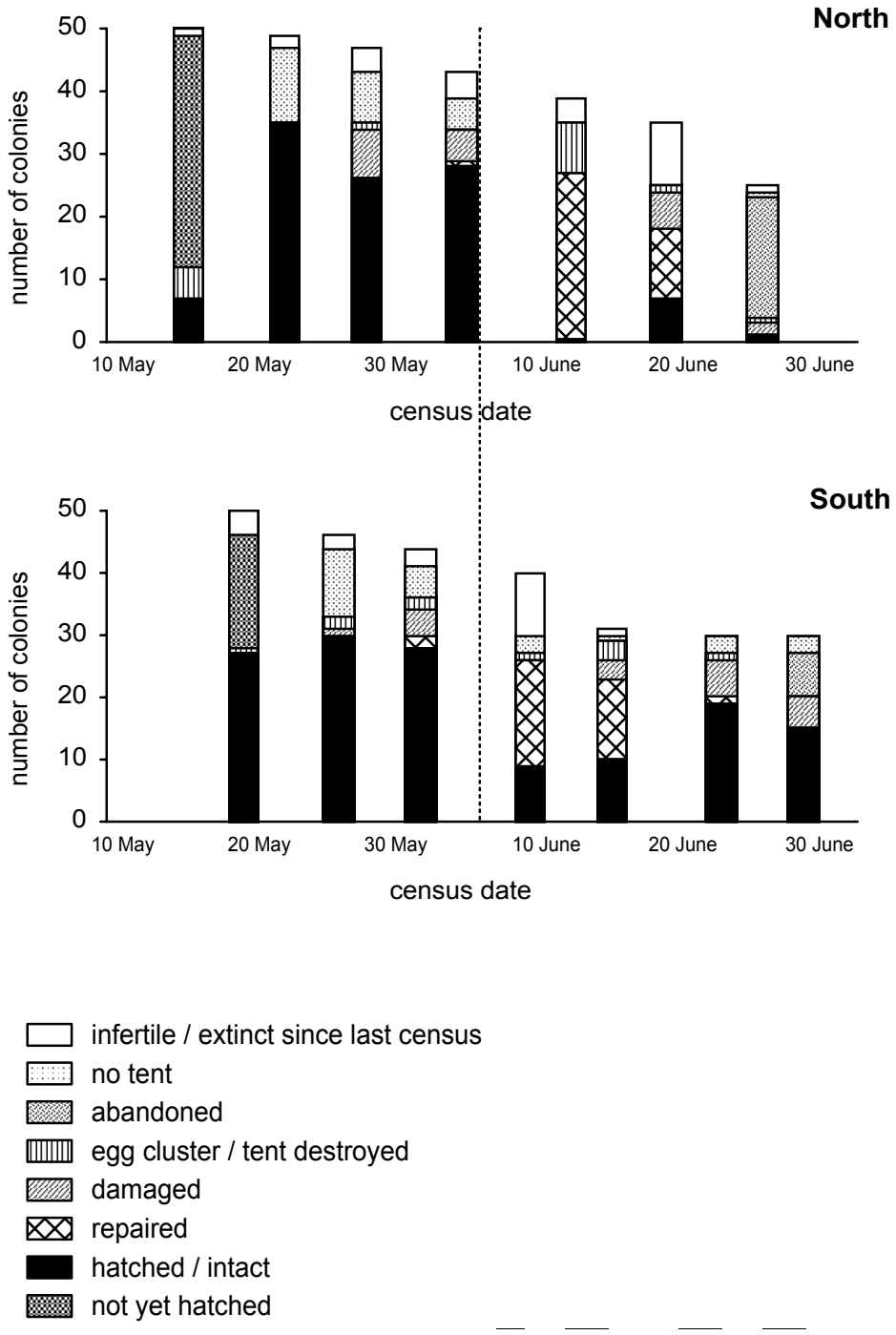

Fig. 6 – Condition of colonies during the seven censuses at each site. The dashed line indicates the start of the heavy rainfalls that severely damaged most tents. North: Colonies at site BB, South: Colonies at sites HL, HP, PB (see Fig. 1).

In contrast to our findings, the *M. americanum* population experienced significant early-instar mortality but negligible mid- to late-instar mortality. Overall, one third of the *M. americanum* colonies was destroyed by the third to fourth instar but no colony became extinct later on. We assume that the relatively higher mid-instar mortality of *E. lanestris* in our study was predominantly due to the damage of the tents after the heavy rainfalls on June 6, which facilitated access of predators to the caterpillars and led to the extinction of whole colonies. Surprisingly, climatic conditions did obviously not contribute to mortality in *M. americanum*, maybe because of evenly spread precipitation without distinct peaks in the year of Costa's (1993) study (i.e. 1990).

Rain seriously affected the constitution of the silk which became brittle leading to large ruptures and holes at the edges of the tent. Additionally, the multiple layers of the tent stuck together after being soaked by heavy rain, which forces the caterpillars to stay outside. Both factors may have contributed to higher mortality through predation following the damage of the tent.

Climatic factors may also have severely contributed to first instar mortality. The five day period (15-20 May) with cool weather (daily maximum <20°C) and with sometimes substantial precipitation (Fig. 2) most likely constrained mobility of the small caterpillars and retarded the construction of the initial tent just after hatching. Under laboratory conditions (ca. 20°C constantly) without precipitation caterpillars build a tent, in which all caterpillars may hide, within 1-2 days after hatching (C. Ruf, pers. obs.). Besides the direct impact of rain and associated low temperatures which retard mobility, foraging, and digestion (Ruf & Fiedler 2002a), it is very likely that those groups that were not able to build a tent fast enough were decimated by invertebrate predators. As a consequence, too few individuals (10-20) were left which were not able to construct a tent any more, and those groups were very likely to become extinct.

The impact of climatic conditions on the abundance of insects has been analyzed for temperate zone butterflies (Roy et al. 2001), often showing positive associations with low rainfall. Furthermore, the impact of extreme weather events (e.g. rainstorms) on butterfly populations was shown in a number of case

histories, although by their very nature such events lack reproducibility (Dennis & Bardell 1996). Although similar long-term studies for moths hardly exist, it is most likely that climatic extremes have the same consequences of larval populations of moths.

Mortality rates of social caterpillars vary enormously among different studies: In two other *Malacosoma* species colony mortality was 40% or more than 95%, respectively (Shiga 1979 cited from Fitzgerald 1995, Filip & Dirzo 1985). While our and other studies on social, tent-building caterpillars focused on colony mortality only and not on mortality of individuals, the latter is probably several times higher.

In the tent building pine processionary *Thaumetopoea pityocampa*, Schmidt et al. (1990) calculated a mean reduction by 62% of the individuals from the mean number of eggs in a cluster to the mean number of mid-instar caterpillars per colony counted in the field. Unfortunately this study disregarded those colonies that had been completely destroyed between egg deposition and the detection of the colony. Another study on web-building caterpillars of *Hyphantria cunea* found mortality rates of 77-100% during the fourth and fifth stadium by birds and wasps (Morris 1972). In a recent study Myers (2000) showed that survival of western tent caterpillars (*Malacosoma californicum*) varied with the natural 6-11 years periodicity of this species. Highest survival rates were measured in early stages of the population increase and survival was lowest during the population decline.

We did not control explicitly for the impact of parasitism and virus or bacterial infections on the survival of colonies but no colony showed obvious signs of strong parasitism (many larval carcasses on the outside of the tents) or disease (heterogeneous multi-instar colonies, shrunken dead caterpillars). Nevertheless, both aspects of mortality may have had a low effect on the colonies.

The possession of an intact tent emerged as the strongest predictor of colony success. Overall, 71% of colony failures were related to the loss of the tent. The functions of the tent are numerous: First of all, the tent facilitates social thermoregulation and enables the caterpillars to elevate their body temperatures high above ambient temperature (Joos et al. 1988, Breuer &

Devkota 1989, Fitzgerald & Underwood 2000, Ruf & Fiedler in press). Besides their thermal function tents of caterpillars serve other purposes like facilitating of mass assembly and recruitment to food (Fitzgerald & Peterson 1983), or reduction of water loss (Wellington 1974). Furthermore, the tent is thought to transmit vibrations to ensure cohesion of group members and to help to keep caterpillars behavior synchronous (Fitzgerald & Costa 1999). Since caterpillars often rest on the surface of the tents, these structures are expected to be only marginally effective in reducing the overall impact of predators and parasitoids (Fitzgerald 1995 and references therein), but sophisticated studies controlling for the sole effect of the tent as a refuge from invertebrate predators are missing so far. We suppose that tents may serve as a shelter for at least part of the time because many invertebrates (e.g. bugs, wasps, ants) do not enter the structure as long as it remains physically intact (C. Ruf, pers. obs.). As a consequence, the destruction of the tent thus means the simultaneous loss of multiple functions enhancing development and communication among the caterpillars which may be the reason for the increased mortality rate among individuals and finally to the complete extinction of a colony.
Silken tents are a costly investment for herbivorous caterpillars (Berenbaum et al. 1993, Craig et al. 1999, Stevens et al. 1999). The importance of the tent is obvious since *E. lanestris* caterpillars repaired the tent whenever it had been destroyed but normally did not leave it. The only cases where a replacement of the tent was observed were associated with the expansion of an already existing *Yponomeuta* web, thus saving costs for a completely new tent.

In a meta-analysis comparing published survival curves for gregarious and solitary Lepidoptera and Symphyta, Hunter (2000) showed that gregarious caterpillars are less likely do die during the earlier instars than solitary caterpillars, but mortality rates rise during the later development (last, solitary instars, pupal phase). Overall, in the comparative analysis generation survival (from egg to adult) was hence not significantly different between solitary and gregarious species whereas the timing of mortality differed between the two classes.

It is noteworthy in this context that in the Lepidoptera there is a continuum from solitary life habits to gregariousness (Costa & Pierce 1997). The duration of the period over which larval cohesion persists as well as the percentage of eggs a female lays in a single cluster vary enormously across species. Hence, there is an important aspect of larval mortality concerning the fitness of the females that remains to be noted when comparing solitary, temporarily gregarious, and highly social caterpillars. Although mortality rates of solitary Lepidoptera larvae may be generally higher during the early larval phase, in all probability at least a few individuals of a female's offspring will escape predation and survive. The same is true for species that distribute the share of risk and lay several smaller egg clusters. In highly social caterpillars where tent-building can only be achieved by large numbers of cooperating siblings colony mortality reduces a female's individual fitness to zero even though mean survival may be high on the population level. Thus, there must be a strong trade-off of tent-building in highly social Lepidoptera between high benefits, primarily by developmental advantages, and high costs due to the risk of complete reproductive failure.

Most of the tents the authors found in the field contained more than 200 individuals. Since the potential fecundity of an *E. lanestris* female is about 300 eggs (C. Ruf, unpubl. data) relative survival seems to be very high once the hatchlings have established their tent and no extreme weather events influence the caterpillars' development. Obviously, in *E. lanestris* the benefits of sociality outweigh the high risk of losing the whole offspring.

The field study presented here is one of the first of its kind to demonstrate the costs of social tent-bound life-habits in gregarious caterpillars. We showed how important a functional, intact tent is for colony survival. In addition to the many laboratory studies that revealed advantages of tent-living for thermoregulation and development, possession of a tent thereby emerged as the single most important predictor of colony survival. This dependence on a silk tent turns into a disadvantage when the tent is destroyed by extreme weather conditions. This means that tent-building as a survival strategy will be selected against whenever the risk of such catastrophes is too high, since each nest destruction is related to complete reproductive failure of the female or very likely of both

parent moths. It will be interesting to see whether with the ongoing global changes in climate, and the associated increasing risk of heavy spring rains in Central and Western Europe, the distribution of *E. lanestris* might change. Climate-related distributional changes are well under way for many Lepidoptera with complex thermal requirements (Kuchlein & Ellis 1997, Hill et al. 1999, Parmesan et al. 1999).

Acknowledgements

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Chapter 11

Larval sociality in three species of central-place foraging Lasiocampidae: A comparative survey

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Abstract

We studied three species of Lasiocampidae with social, tent-building caterpillars in Northern Bavaria, viz. *Eriogaster lanestris*, *Eriogaster catax*, and *Malacosoma neustria*. We used key life-history data (number of larval instars, sizes and weights of eggs, caterpillars, and moths, size of egg clutches) as well as behavioral data (activity patterns, tent-building behavior, trail following behavior) for a comparative study. Although larvae of all three species are active only in spring, show overlapping habitat requirements, and use the same major host-plant (*Prunus spinosa*) with only minor differences in phenology, they show markedly different life-history and behavioral strategies.

E. catax lays comparatively few but large eggs while *E. lanestris* lays more but smaller eggs. *M. neustria* lays the smallest eggs but large clusters. *E. lanestris* caterpillars build a large tent with an accessible interior while those of *E. catax* build a small tent that is only used as a resting and molting platform. *M. neustria* shows a flexible behavior, may abandon the primary tent and build a new one several times. *M. neustria* colonies also subdivide and reunite regularly while *Eriogaster* colonies stay together until larvae become solitary. In *E. lanestris* all tentmates of a colony are highly synchronized while foraging or resting. Instead, in *E. catax* small subgroups leave the tent for foraging while at every time the majority rests on the tent. *M. neustria* caterpillars forage more or less individually and only synchronize by night. Results are discussed in relation to other

species of the genera *Eriogaster* and *Malacosoma* and with regard to the evolution and diversification of caterpillar sociality.

Introduction

Social phenomena in animals have interested scientists ever since. In insects, this particularly holds for eusocial Hymenoptera (ants, bees, wasps) that share common features like overlapping generations, cooperative brood care and reproductive division of labor (Costa 1997). Social systems in Lepidoptera larvae are far less complex. There are no parent-offspring interactions and caterpillars only build up single-generation cohorts that dissolve when the larvae mature. Furthermore, genetic factors promoting social cooperation like kin selection (Hamilton 1964) do not play a role (Costa & Ross 1993). Nevertheless, there is considerable communication among group members, resulting in complex behavioral cooperation like communal foraging, defense and nest building (for an overview see Costa 1997).

Social caterpillars are commonly classified in three categories with regard to their foraging strategies (Fitzgerald & Peterson 1988). Patch-restricted foragers only feed within their loosely spun webs, whereas nomadic foragers do not build a three-dimensional web at all. Instead, they spin at most two-dimensional resting mats, move from one site to the next, and feed and rest in groups. Only few species use a permanent home base, usually in the form of a silken densely woven tent or resting platform, throughout the social phase of the larvae's life (so-called central-place foragers, CPF). Since those tents do not include any resources caterpillars have to leave their home-base for every food intake. As a consequence, resources become more and more depleted and patchily distributed during the caterpillars' life. Moreover, tents of CPF species serve as multifunctional structures for thermoregulation (Joos et al. 1988, Ruf & Fiedler 2002b), coordination of colony activity (Fitzgerald & Costa 1999) and probably as a shelter against predators. This leads to the assumption that CPF species should have evolved more complex societies based on communicative systems that facilitate cooperative foraging and other adaptive interactions as compared to any other social Lepidoptera.

In Central Europe there are only few moth genera with social CPF caterpillars. Besides some species of processionary caterpillars (*Thaumetopoea* spp., Notodontidae) CPF social systems are here only found in the lasiocampid genera *Eriogaster* and *Malacosoma*. Although some older descriptive publications deal with the general natural history of some species of these taxa (e.g. Balfour-Browne 1933, Carlberg 1980, Tutt 1900), there is a nearly complete lack of quantitative, basic data with the exception of few species of economic interest (Fitzgerald 1995 and references therein).

This paper has two objectives: Firstly, it shall add to our knowledge of the biology of three CPF species with overlapping habitat requirements which use the same major host plants (Ebert 1994). These species include the small eggar, *Eriogaster lanestris* L., its scarce relative *Eriogaster catax* L. and the common lackey, *Malacosoma neustria* L. It is our special concern to enlarge current knowledge of the highly endangered species *E. catax*, since the only recent publication on this species (Bolz 1998) lacks concrete quantitative data and observations reported there are frequently inconsistent with our data.

Secondly, we use a comparative approach for elucidating ecological strategies that may explain niche segregation among these three species. We specifically focus on the use of the tents, on strategies for daily activity, and also deal with communicative aspects (trail following behavior). Based on these data comparisons with other species shall add to our understanding of the evolution and diversity of social phenomena in the genera *Eriogaster* and *Malacosoma*.

Material and Methods

Geographical range of study species: All three species have a Palaearctic distribution but ranges do not overlap completely. *M. neustria* shows the widest distribution and can be found almost throughout the Palaearctic region (Table 1). *Eriogaster lanestris* also shows a wide Palaearctic distribution but is absent from the Mediterranean region. In contrast, *E. catax* has a more restricted distribution and is absent from the northern countries in Europe. In Germany, the northernmost populations today occur in Northern Bavaria.

Table 1. Selection of life-history traits that characterize the three species investigated. Data on phenology and hostplants from Ebert 1994. Data on geographical range: Ebert 1994, de Freina & Witt 1987, Dubatalov & Zolotuhin 1992, ProNatura 2000.

	E. lanestris	*E. catax*	*M. neustria*
Geographical range	Europe (except Mediterranean and Tundra), N Caucasus, Kazakhstan, S Siberia, Central Jakutia	N Spain, Central Europe (except North), Ural, Asia Minor	Nearly whole Palaearctic: Europe, Mediterranean Islands, N Africa, Asia, Japan
Overwintering stadium	Pupa	Egg	Egg
Larval phenology (sequence of hatching in spring)	third	first	second
Moths flight season	Spring (March-April)	Autumn (Sep.-Oct.)	Summer (June-Aug.)
Major host plants	*Prunus spinosa*, *Crataegus* sp., *Betula* sp., *Tilia* sp., *Salix* sp.	*Prunus spinosa*, *Crataegus* sp., *Quercus* sp.	*Prunus spinosa*, *Prunus domestica*, *Malus* sp., *Betula* sp.

Habitat requirements: Although all species may share the same habitat (A. Geyer, pers. obs.), habitat requirements are not identically but overlap considerably. *M. neustria* uses a wide spectrum of habitats like deciduous forests, hedgerows, parks, and orchards (Ebert 1994). *E. lanestris* is more restricted to warm and xeric habitats and often lives on blackthorns on south-facing slopes. The primary habitats of *E. catax* are warm, relatively humid, sparsely-wooded, open forests but it may also occur on blackthorn hedgerows (Ebert 1994).

General phenology of the study species: The three species are all strictly univoltine with larvae occurring in spring. However, life-history traits differ greatly with respect to the overwintering stadium, the flight season of the moths and the precise phenology of the caterpillars (Table 1). The phenology of blackthorns is characterized by an early phase of blossoming, withering, and delayed budbreak about one week after blossoming (Bartels 1993). *E. catax* caterpillars can regularly be found before leaf budbreak when only blossoms and small leaf buds are available as food (in our study area around the end of April or the beginning of May). Caterpillars of *Malacosoma neustria* hatch in synchrony to leaf budbreak (ca. 10 days later) and *E. lanestris* caterpillars hatch

shortly after budbreak when leaves are not yet fully developed (in the middle of May, A. Freese & C. Ruf pers. obs.).

Origin of the animals investigated: All animals stemmed from various sites in Northern Bavaria. Colonies of *E. lanestris* were collected in 1999. The animals used for this study belonged to the third laboratory-bred generation of these animals. Inbreeding was avoided by only breeding with males and females from different colonies (1000-1500 individuals each year). Colonies of *M. neustria* were bred in the laboratory (first generation). They all stemmed from males and females from one colony (ca. 150 individuals) which was collected in 2001. Colonies of *E. catax* were either collected in the field in the second larval stadium or were bred in the laboratory from animals collected in 2001.

Determination of the two Eriogaster species in young colonies: For an early identification of field colonies or for the identification of abandoned tents, it is necessary to reliably identify egg clusters and young caterpillars. The egg cluster of *E. lanestris* is usually coiled around a small twig several times whereas that of *E. catax* is more flatly spread along a bigger branch. Additionally, the hairs deposited by the mother from her anal tuft on the eggs look very different: The hair of *E. catax* is very smooth and more checkered (darker at the base, paler at the top, appearing silver). The most striking feature of the whole egg cluster is a dark brownish ridge along its longitudinal axis. In *E. lanestris* the color of the hair appears uniformly gray and the structure of the hair cover is less smooth.

Immediately after hatching caterpillars of *E. catax* have reddish setae whereas those of *E. lanestris* are white. Differentiation is easy after the first molt when *E. catax* caterpillars show the characteristic tufts of hair on the second and third thoracic segment which are not yet visible after hatching.

Measuring activity patterns: Activity patterns of the caterpillars were monitored in the laboratory (environmental cabinet, L:D 14:8h, 18°C) and under near-field conditions (roof of university building) by using light barriers attached to both sides of a rod leading from the tent to a bunch of blackthorn twigs in a vase. Thus, the number of caterpillars crawling between the plant and the tent was

automatically registered in 1-minute intervals. Besides, ambient air temperature was measured in the shade nearby the tents. Details of the methods can be found elsewhere (laboratory: Ruf et al. 2001b, field conditions: Ruf & Fiedler 2002a).

Size and weight measurements: Body weights (fresh weight) were measured to the nearest milligram on an electronic balance (Sartorius BL 150 S) except for the weights of tiny newly hatched caterpillars that were pooled to a group of several individuals and weighed to the nearest 0.01mg (Sartorius MC 210 P). Moths were weighed immediately after their wings were fully expanded prior to the excretion of the meconium and therefore prior to flight or egg deposition. To measure the weight of full grown larvae we chose caterpillars that looked as large as the larvae would grow according to our experience. This somewhat inaccurate method had to be used because *Eriogaster* and *Malacosoma* caterpillars cease feeding several days before pupating and shrink to less than half of their maximum size before they start to build their cocoon. Since caterpillars were not sexed it is likely that we measured females only, which grow larger than males. Maximum length of full grown caterpillars was measured with a caliper rule when caterpillars sat fully stretched and undisturbed on a branch.

Widths of head capsules were measured with the help of a stereo microscope using a calibrated scale at a 40-fold magnification. For instars L1 to L4 we used head capsules which were thrown off during molting. Head capsules of last instar caterpillars were measured at the living caterpillar since the head capsule is destroyed during the pupal molt. Thus, due to larval movements inaccuracy of measurements is higher for the last instar.

Cross-section dimension and length of eggs were measured at 70-fold magnification. Egg volume was calculated by first taking photographs of the eggs. The outlines of the eggs (halves) were then digitalized and the volume was determined by calculating a rotational body of this shape with the help of a software (FormZ, Ver. 2.9, AutoDesSys).

Trail following experiments: Trail following abilities have been shown for *M. neustria* (Peterson 1988) and *E. lanestris* (Ruf et al. 2001a). Both species release their trail marker on the ventral side of the last abdominal segment. Nothing is yet known about the trail following abilities of *E. catax*, and there are no studies on communication across species boundaries.

In order to gain defined trails with the natural trail marker a 10 × 6cm-sheet of paper was folded along both diagonals (see Fitzgerald & Underwood 1998a,b for method). Parts of the creases were marked 1cm in one direction and 3cm in the other direction starting from the crossing point. To obtain artificial trails, the venter of the tip of the abdomen of one caterpillar was dragged three times along one of the creases between the markings. The sheet was then unfolded and another caterpillar was placed on the paper with its head between the short markings of the two creases, now building a slight ridge. Swinging its head from side to side the caterpillar touched both creases and could therefore find the prepared trail. A positive response was scored when the caterpillar followed the trail to the mark within three minutes after it had started to search for a trail.

Other *Malacosoma* species (*M. americanum* and *M. disstria*) as well as caterpillars of *E. lanestris* follow trails prepared with the steroid 5β-cholestane-3-one (Fitzgerald 1993, Ruf et al. 2001a). In order to test whether caterpillars of *E. catax* and *M. neustria* show trail following behavior in response to this substance as well, artificial trails were prepared by laying out the steroid solved in hexane in an S-shape on a filter paper (Sigma Chemical Company, Coprostane-3-one, concentration: ca. 4×10^{-10}g/cm). This concentration is sufficient to release a strong trail following response in *E. lanestris* (Ruf et al. 2001a). One L2 caterpillar was placed at the end of the trail and the behavior was observed. The test was replicated with three caterpillars of each species.

Use of the tent: We observed tent building behavior and the use of the tents for the three species under field and laboratory conditions (exception: *E. catax* only field data). In particular, we noted where precisely the larvae rested, if the same tent was used throughout larval development and how the tent was enlarged by cooperative spinning behavior.

Results

Clutch size, volume of eggs, and body size: Females of all three species always laid their full egg complement into one egg batch. The size of egg clusters varied between species. Egg clusters of *E. lanestris* were the largest but did not differ significantly compared to *M. neustria* (Table 2). Egg clusters of *E. catax* proved to be significantly smaller. Comparing the eggs of the three species reveals that they are shaped quite differently. Thus, despite only minor differences in length and width, egg volumes of *E. catax* are ca. 50% larger than those of *E. lanestris* and are three times larger than those of *M. neustria*. The different volume of eggs is also reflected in the different weight of hatching caterpillars that is also largest in *E. catax* (Table 3).

Table 2. Number of eggs per clutch. Different letters indicate significant differences (Kruskal-Wallis ANOVA: $H(2df)=54.6$, $p<0.001$, followed by Tukey-Kramer post-hoc test, $p<0.05$).

Species	Origin	N	Number of eggs in cluster		
			mean±SD	min	max
E. lanestris	Laboratory bred	20	323±71[a]	210	470
E. catax	Field	39	183±40[b]	55	293
M. neustria	Laboratory bred	20	292±40[a]	210	350

There are no significant differences between the body weights of fully grown larvae of either *E. catax* or *E. lanestris*, whereas *M. neustria* attains a significantly lower maximum body weight (Kruskal-Wallis ANOVA, $H(2df)=37.9$, $p<0.0001$).

Table 3. Size and weight of *Eriogaster lanestris*, *E. catax*, and *Malacosoma neustria*. * mean weight calculated from pooled data. Different letters within a column indicate significant differences between larval stages (width of head capsule: Scheffé test after ANOVA). Different letters within a row mark significant differences between species (body weights: Tukey-Kramer test after Kruskal-Wallis ANOVA).

Parameter		N	*E. lanestris*	*E. catax*	*M. neustria*
Egg: cross section dimension		15	0.75 ± 0.05	0.73 ± 0.02	0.64 ± 0.03
Egg: length		15	1.17 ± 0.07	1.56 ± 0.04	1.07 ± 0.08
Egg: volume (calculated) [mm³]		-	0.52	0.73	0.25
Width of head capsule [mm]	L1	20	0.55 ± 0.01^{a}	0.56 ± 0.02^{a}	0.45 ± 0.01^{a}
	L2	20	0.96 ± 0.02^{b}	0.89 ± 0.02^{b}	0.72 ± 0.04^{b}
	L3	20	1.60 ± 0.06^{c}	1.46 ± 0.04^{c}	1.28 ± 0.07^{c}
	L4	20	2.56 ± 0.08^{d}	2.17 ± 0.06^{d}	2.17 ± 0.08^{d}
	L5 (ultimate)	20	3.26 ± 0.19^{e}	3.34 ± 0.10^{e}	3.47 ± 0.18^{e}
	L5 (penult.)	19	–	–	2.66 ± 0.10^{f}
	L6	16	–	–	3.77 ± 0.18^{g}
Body length (maximum) [mm]		-	54	55	58
Body weight of hatching caterpillar [mg]		(15-20)	0.43*	0.49*	0.21*
Body weight of full grown larva [mg]		20	1278 ± 112^{a} max: 1530	1294 ± 51^{a} max: 1390	920 ± 59^{b} max: 1029
Body weight of adult moth [mg]	male	10-20	236 ± 24	no data	167 ± 25
	female	10-20	434 ± 63	no data	378 ± 54

Number of larval stages and width of head capsule: Eriogaster lanestris and *E. catax* both passed through five larval stages which could clearly be separated by the width of the head capsule (Table 3; *E. catax*: F(4df)=7413, *E. lanestris*: F(4df)=2830, both p<0.0001, Scheffé post-hoc tests, all p<0.0001). In contrast, the number of larval stages was variable in *Malacosoma neustria* which had either five or six stages. Although the widths of the head capsule were all significantly different (F(6df)=2658, p<0.0001, Scheffé post-hoc tests: all p<0.0001) it is not possible to unambiguously recognize caterpillars as either fifth (if ultimate) or sixth instar, since there was some overlap in the data. The sixth instar in *M. neustria* did not depend on the sex of the caterpillar. Eight of 18 caterpillars passing through a sixth instar proved to be males and ten proved to be females (χ^2(1df)=0.2, n.s.).

Emergence and sex-ratio: Emergence rates in the flight season following pupation were generally very high in *M. neustria*, whereas they were distinctly lower in the two *Eriogaster* species as they show a considerable proportion of delayed hatching (Table 4). The sex-ratio was balanced in *E. lanestris* and *M. neustria*. In *E. catax* subitaneous hatching in 2001 indicated a female-biased sex ratio but when moths from the same cohort were considered, which emerged in the flight season of 2002 (i.e. one year later) the overall sex-ratio was unbiased. Delayed emergence in *E. lanestris* was not taken into account since those moths will soonest hatch in 2003.

Table 4. Sex-ratio of the three species investigated. n.s.= not significant. Delayed hatching was disregarded in *E. lanestris* and is not known from *M. neustria.*

Species	Origin	Eclosion	Males	Females	Proportion m : f	χ^2(1df)	p
E. lanestris	Breed. 2001	86% (430 of 500)	105	95	1.1 : 1	0.50	n.s.
E. catax, subitaneous	Field 2001	72% (153 of 214)	58	95	1 : 1.6	8.95	<0.01
E. catax, delayed		8% (17 of 214)	16	1	16 : 1	13.24	<0.001
E. catax, total		79% (170 of 214)	74	96	1 : 1.3	2.85	n.s.
M. neustria	Field 2001	97% (146 of 151)	60	72	1 : 1.2	1.09	n.s.
M. neustria	Breed. 2002	100% (167 of 167)	86	81	1.1 : 1	0.15	n.s.

Use and construction of the tent: Although all species build a densely woven tent, the use and size of the tent differed markedly (Table 5). *E. lanestris* colonies built one tent, usually right around the egg mass. Only very rarely, when the original tent had been destroyed through heavy rainfall, a secondary tent was constructed (Ruf & Fiedler, submitted). The tents were continually expanded by adding new silk layers and finally became large in size (i.e. about 750cm³ during the last instar for an average sized tent). The tents had an accessible interior. Accordingly, caterpillars changed their position on and in the tent frequently to achieve optimal body temperatures (Ruf & Fiedler 2002b).

Tents were usually built at the outermost end of branches with optimal exposure to solar radiation. Caterpillars left the tent only shortly before pupation.

Table 5. Use of tent in *E. lanestris*, *E. catax* and *M. neustria*.

Species	N observations		Exposure on blackthorn	Use	Number of tents	Duration of use
	Field	Laboratory				
E. lanestris	>100	>50	Outer margin, sun exposed	Surface and interior	1	Until end of last (=5th) instar
E. catax	>100	10	Set back, sun exposed	Surface only	1	Until beginning of penultimate (4th) instar
M. neustria	10	>50	Outer margin, sun exposed	Preferably surface	variable	Until beginning of 5th instar

In contrast, caterpillars of *E. catax* also built only one tent, usually above the egg mass, but which was comparatively small in size (roughly 300cm³). Building a secondary tent at a new site due to the local exhaustion of the blackthorn was scarce (less than 5% of the observed tents, total N=150). The tent was exclusively used as a resting platform because it was more flat and had no accessible interior. We never observed larvae of any instar staying inside the tent. Nevertheless, tents are three-dimensional structures and not just a silk mat. Caterpillars usually rested in tightly aggregated groups on its surface or rested on its underside when solar irradiation was too strong or during phases of rain. The tent was always situated within the blackthorn bushes woven close to the branching point of several thick branches (diameter = 5.8±2.6cm, N=33). Despite the comparatively deeper location of the tents in the bushes, tents were predominantly found on sun exposed positions, with 46.4% of the tents occurring in southern directions (SE, S, SW), and another 21.1% of the tents situated on the top positions of the plants. Only one third (32.5%) of the tents were exposed to western, eastern, and northern directions (N=114). Caterpillars left the tent at the beginning of the fourth (=penultimate) instar, usually 1-2 days after molting. By then, they dropped off the tent and crawled away.

Caterpillars of *M. neustria* showed a more flexible behavior in terms of tent building. They sometimes built only one small tent which was inhabited until the end of the fourth or the beginning of the fifth instar. Very often (>80% of cases, N=50 colonies), however, the primary tent was abandoned and caterpillars built a new tent elsewhere. Colonies regularly subdivided into smaller groups but also reunited later on. Changes of the home base were not bound to molting phases. The tent was always situated sun-exposed at the end of the branches. Tents reached a maximum size of roughly 300cm³. Under field conditions as well as in the laboratory, caterpillars were predominantly found on the surface of the tent. Only rarely, single individuals hid inside the structure. Besides, caterpillars could also often be found hanging in tight aggregations with the head downwards on twigs away from the tent.

Activity patterns: Figure 1 shows exemplary recordings of typical foraging patterns of the three species.

Eriogaster lanestris: Activity patterns of *E. lanestris* have been described elsewhere in detail (Ruf et al. 2001b, Ruf & Fiedler 2002a). Foraging bouts are highly synchronized and always include all caterpillars from a colony (apart from those just about to molt). In addition, they are clearly distinct from resting phases, when all caterpillars rest in or on the tent. The number of foraging bouts per day depends on temperature and varies between one and nine.

Eriogaster catax: Under laboratory conditions we recorded a more or less regular, pulsed distribution of signals during the course of a day (Fig. 1, Fig. 2: Lab1, Lab2). Thus, caterpillars left the tent and returned to it in rather synchronized groups. Resting times between activity bouts were only weakly differentiated. It is noteworthy, however, that these foraging groups did never include all caterpillars from a colony. Instead, at any time the majority of caterpillars stayed on the tent, always in a tightly packed group, usually forming a flat circular clump. Under field conditions activity patterns were similar, but there were fewer movements between the tent and the plant during the night time hours when temperature fell below about 15°C (Fig. 2: Fi1, Fi2).

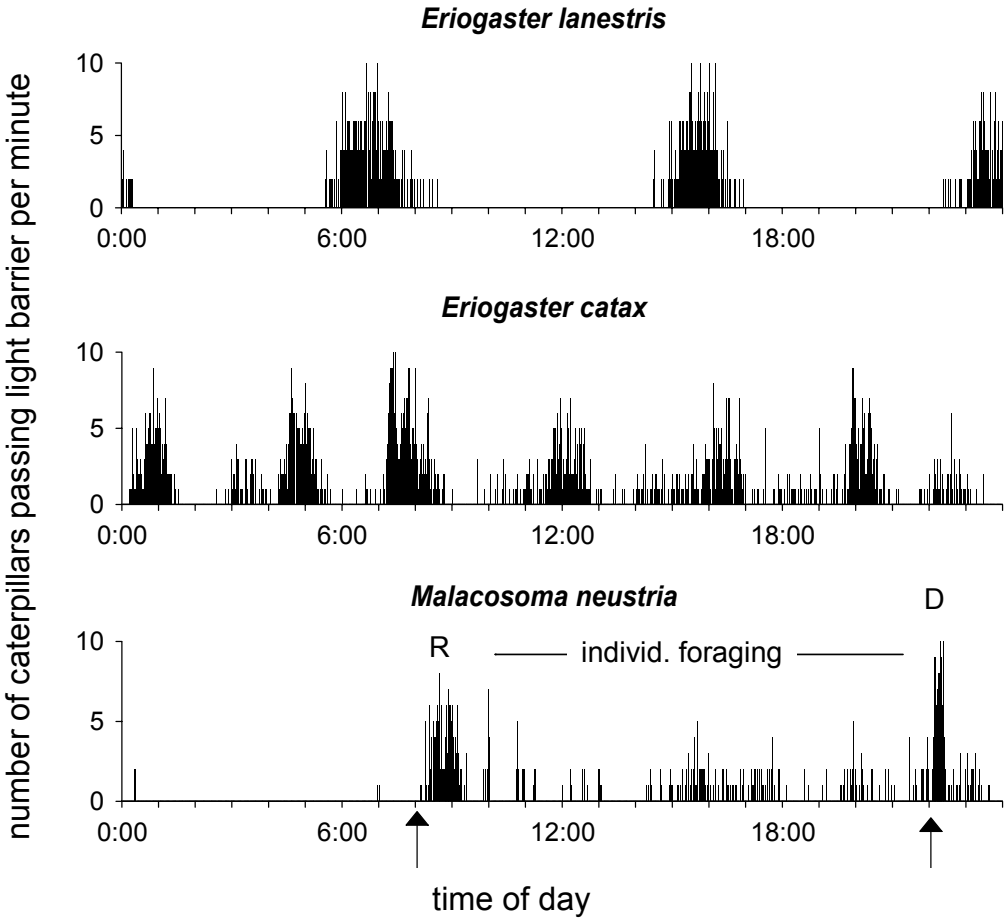

Fig. 1 – Exemplary recordings of typical foraging patterns of caterpillar colonies under laboratory conditions at 18°C. *Eriogaster lanestris*: Synchronized colony activity. Every peak represents a synchronized foraging bout of all tentmates. *Eriogaster catax*: Synchronized subgroups. One peak represents a more or less synchronized foraging bout of only part of the caterpillars of a colony. *Malacosoma neustria*. Individual foraging by day, nocturnal synchronization. R=return, D=departure from/to nocturnal feeding on plant.

Malacosoma neustria: Foraging patterns of *M. neustria* are the most flexible among the three species investigated. The most striking pattern is the peak of signals at dawn and the almost complete absence of nocturnal signals (Fig. 1). Direct observations revealed that caterpillars of *M. neustria* left the tent in the evening and stayed on the plant during the whole night. They returned to the tent not until the morning which causes a second distinct peak in the automatic recordings (see arrows in Fig. 3). During the day, caterpillars usually returned to the tent after every food intake. In the laboratory foraging activities during daytime were never synchronized and caterpillars foraged individually. In contrast, caterpillars synchronized their activities under field conditions and

showed distinct, albeit widely spread activity bouts at least at low temperatures. The morning return proved to be less synchronized and temporally flexible. Since we have no direct observational data under field conditions for longer periods of time we do not know whether all caterpillars of the colony, or only a fraction, participated in those activity bouts.

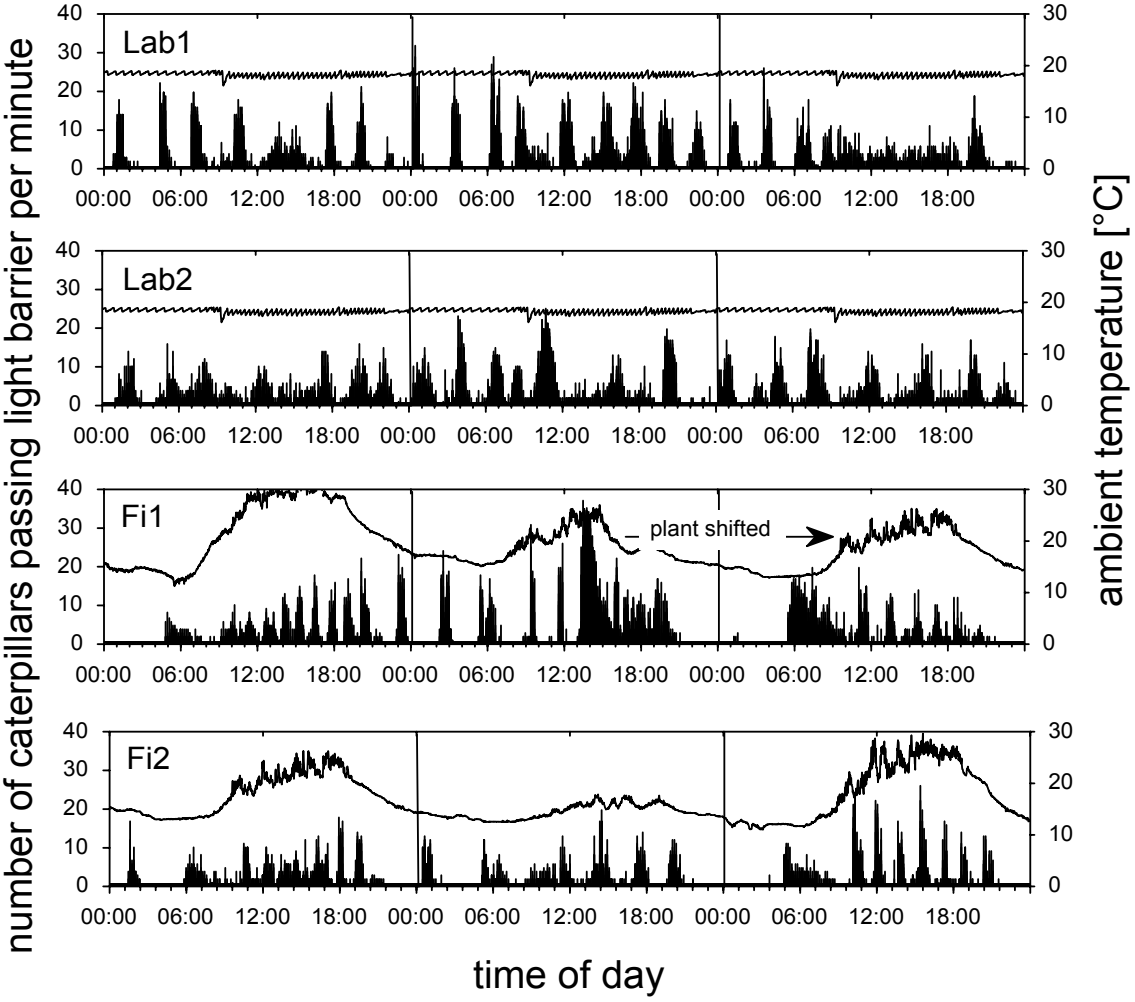

Fig. 2 – Recordings of foraging activities of four different *Eriogaster catax* colonies (3rd instar) during three consecutive days. Lab1, Lab2: in the laboratory. Fi1, Fi2: under field conditions. In colony Fi1 activity patterns were disturbed between day 2 and 3 because the plant was shifted by a storm and caterpillars had no access to the plant anymore.

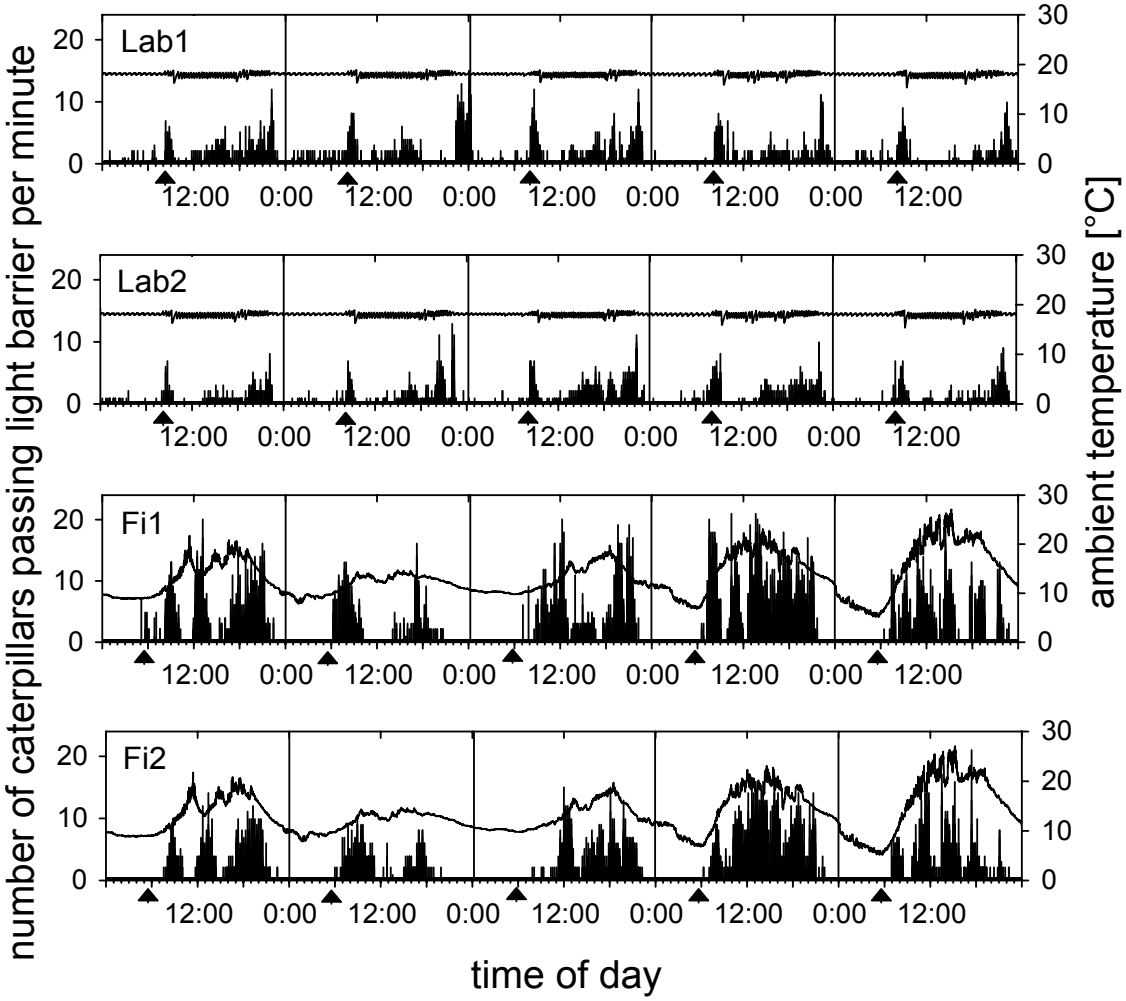

Fig. 3 – Activity patterns of four different *Malacosoma neustria* colonies (4th instar) on five consecutive days. Lab1, Lab2: in the laboratory. Fi1, Fi2: under field conditions. Marks at the time axis denote the time when the light was turned on (environmental chamber = Lab1, Lab2) or the time of sunrise (Fi1, Fi2), respectively.

Trail following: Caterpillars of *E. catax* and *M. neustria* both followed the S-shaped artificial trails prepared with 5β-cholestane-3-one. The same had already been shown for *E. lanestris* (Ruf et al. 2001). *E. lanestris* readily followed trails prepared from surface residues gained from third, fourth (=penultimate) and fifth (=ultimate) instar caterpillars of *E. catax* as well as fourth instar caterpillars of *M. neustria* (Table 6). *M. neustria* only occasionally found trails prepared with surface residues of *E. catax*, when the material was gained from only one caterpillar. If two source caterpillars were used, the majority of *M. neustria* larvae found the trail of *E. catax* as well as those of *E. lanestris*. The ability of caterpillars of *E. catax* to recognize trails of either *E.*

lanestris or *M. neustria* could not be tested since caterpillars refused to search for a trail in the experimental situation.

Table 6. Cross-species attractiveness of the trail marker of three lasiocampid caterpillars (non-choice tests). *caterpillar repositioned up to three times if it crawled away without showing searching behavior. *E. lanestris* and *M. neustria* show reciprocal trail following behavior on trails of the other species and on trails of *E. catax*. *E. catax* caterpillars were not used as test species since caterpillars refused to search for a trail in the experimental situation.

Origin of trail marker	Species tested for response	Trail found	No trail found	χ^2(1df)	p
E. catax L3	*E. lanestris* L3	11	1	8.3	<0.01
E. catax L4	*E. lanestris* L3	11	1	8.3	<0.01
E. catax L5	*E. lanestris* L3	12	0	12.0	<0.001
E. catax L4	*M. neustria* L3	7*	5	0.3	n.s.
2 *E. catax* L4	*M. neustria* L3	10*	2	5.3	<0.05
2 E. lanestris L3	*M. neustria* L3	11	0	11.0	<0.001
M. neustria L4	*E. lanestris* L2/L3	12	0	12.0	<0.001

In choice tests, caterpillars of *E. lanestris* were not able to distinguish their own trails from those of the congeneric species *E. catax*, and even preferred trails of the confamilial species *M. neustria* over their own trails (Table 7).

Table 7. Decision of *E. lanestris* caterpillars (second and third instar) when choosing between conspecific and congeneric, or between conspecific and confamilial trails, respectively.

Trail 1	Trail 2	Choice Trail 1: Trail 2	χ^2 (1df)	p
E. catax L4	*E. lanestris* L4	10:10	0.0	n.s.
M. neustria L4	*E. lanestris* L4	20:6	7.5	<0.01

Discussion

Our data show that despite many similarities in larval life history traits a broad diversity of ecological and behavioral strategies may arise among tent-building Lasiocampidae. Furthermore, closely related (i.e. congeneric) species need not necessarily exhibit more similar strategies than other, more distantly related species of the same family.

Egg size and fecundity

The mean potential fecundity of a female of *E. catax* is less than 60% compared to a female of *E. lanestris* although full grown caterpillars are equal in weight and adult moths are also very similar in size. Obviously, females of *E. catax* adopt a different strategy and lay fewer but larger eggs and also have larger hatchlings. It is generally acknowledged that there is a strong trade-off between egg size and number (Fox & Czesak 2000 and references therein, Fischer & Fiedler 2001). This effect is especially strong for semelparous arthropods that use larval-acquired resources for egg production (i.e. capital breeders: Tammaru & Haukioja 1996). This is true for all Lasiocampidae which do not possess a functional proboscis and totally rely on the larval resources for adult reproduction (e.g. Lemaire & Minet 1999).

The adaptive significance of different egg sizes is a controversial issue. It is generally acknowledged that larger eggs give rise to larger offspring (Fischer et al. 2002, Reavey 1992). Furthermore, many studies on different insects support the hypothesis that larger offspring can often better withstand environmental stresses such as starvation or desiccation (Fox & Czesak 2000, Fischer & Fiedler 2001). In contrast to these findings Reavey (1992), in an extended comparison across species, observed no general pattern in Lepidoptera. Neither were larvae hatching from larger eggs less susceptible to starvation nor more mobile. Nevertheless, it seems reasonable to hypothesize that larger hatchlings are advantageous for *E. catax* since these caterpillars often hatch before budbreak and have to walk considerable distances from their tent to find suitable buds or blossoms for feeding. Furthermore, larger eggs might facilitate to survive cold winter temperatures. Species that overwinter in the egg stage generally have larger eggs which contain greater energy reserves or have a thicker chorion (Reavey 1992 and references therein). Additionally, species that oviposit on hosts with tougher leaves were shown to lay larger eggs (Nakasuji 1987). It seems feasible that feeding on buds requires a relatively larger head size than feeding on young soft leaves which would also select for a higher hatchling weight. Whatever the ecological significance of larger egg and hatchling size may be, in *E. catax* this constrains the size of larval aggregations

that can be attained. It should also be noted that our volume-based estimates of egg size (and thus of investment into eggs) produced more realistic values than would have been possible by simple measures of linear egg dimensions (egg diameter and height, Garciá-Barros 2000). Using linear dimensions only we would have been unable to detect the significant difference between *E. catax* and *E. lanestris*, which are exclusively due to egg geometry.

Comparisons of our clutch size data with literature data are difficult, since most available data lack details about sample sizes. Clutch size for *M. neustria* reportedly varies between 100 and 400 with mean values of about 300 eggs (Tutt 1900) which is consistent with our data. Egg clusters of *E. lanestris* have been reported to contain 150-300 eggs (data from English populations, Balfour-Browne 1933). The number of eggs per egg mass is positively correlated with the mass of the ovipositing female which in turn reflects resource accumulation during larval development (cf. Fitzgerald 1995). Thus, clutch size is typically highly variable within species. This was also true in our samples, where clutch sizes varied 1.6 to 5.3-fold within species. Thus, overall our quantitative data are in reasonable agreement with these published records.

Activity patterns

All caterpillars of the genera *Malacosoma* and *Eriogaster* for whom data are available show a mixture of diurnal and nocturnal foraging (Ruf & Fiedler 2002a and references therein). However, when looked upon in more detail the three species investigated in this study show very different foraging strategies, ranging from temporally flexible, strictly synchronized foraging (*E. lanestris*) to foraging in more or less synchronized subgroups (*E. catax*) or even individual foraging with only nocturnal synchronization (*M. neustria*). Besides, the North American species *Malacosoma americanum* shows another more strictly scheduled foraging pattern with three foraging periods per day, one each during sunrise and sunset, and one around noon (Fitzgerald et al. 1988). All other central-place foraging species for whom data are available rely on strictly nocturnal foraging (see references in Ruf & Fiedler 2002a). Interestingly, non of these nocturnally foraging species has spring feeding larvae. Temperate zone

spring species typically face cold temperatures at night but often high radiation during the day. It is therefore reasonable to suppose that diurnal activity is associated with the need to thermoregulate and to make use of elevated body temperatures during the daylight hours (cf. Casey et al. 1988, Ruf & Fiedler 2002b). Probably, this diurnal foraging activity is then further shaped by biotic factors. For example, coordinated and synchronized foraging may be advantageous to minimize conspicuousness to visually hunting predators but may be useless when food patches are too small or too scattered in distribution to support the whole colony at the same time (as in the very early bud-feeding *E. catax*). Variation of activity patterns within and across species indicates that there is ecological as well as evolutionary plasticity of this trait in tent-building social Lasiocampidae larvae. Automatic long-term recording of foraging activities with electronic light barriers again turned out to be necessary to fully uncover this variation (see Ruf et al. 2001b).

Trail following

Although the trail following abilities of caterpillars of *E. catax* could only be tested on artificial trails it seems reasonable to assume that all three species tested use chemically similar trail markers. This result is not surprising. In contrast to sex pheromones that have a direct impact on reproduction and fitness, trail pheromones are far less species specific and studies on ants have shown that even phylogenetically unrelated species may use the same trail pheromone (Hölldobler & Wilson 1990).

It is noteworthy, however, that caterpillars of *E. lanestris* even prefer trails of *M. neustria* over their own trails and that *M. neustria* only shows trail following behavior on *Eriogaster* trails if the trail is prepared with surface residues from two caterpillars. Possibly, *M. neustria* caterpillars produce higher quantities of the pheromone. Thus, trails may be stronger compared to *E. lanestris* trails. This assumption is also consistent with the observation that *M. neustria* caterpillars did not react to trails prepared from surface residues of only one *Eriogaster* caterpillar because this trail is probably below the threshold level. Peterson (1988) showed that in *M. neustria* the frequency of marking declines

rapidly with caterpillar order with the tenth caterpillar passing a trail showing a probability of marking of less than 10%. Thus, trails obviously reach a high level of acceptability after only few individuals have passed a trail which suggests that pheromone quantities applied per caterpillar are relatively high.

Fitzgerald & Edgerly (1979) also showed that caterpillars of *M. americanum* preferred trails of *Malacosoma disstria* to trails produced by unfed *M. americanum* caterpillars but preferred their own trails if caterpillars used to prepare the trails were satiated. Again, quantitative effects may be responsible for this effect. It was also proposed that qualitative differences may play a role but these assumptions remain speculative until the chemical composition of the trail markers is fully analyzed.

Tent building and tent use

Many studies have shown the thermoregulative advantages of silken tents (e.g. Joos et al. 1988, Breuer et al. 1989, Ruf & Fiedler 2002b). All species that have been investigated so far preferably build their tents in a sun-exposed position on the bush or tree and use both, the tent's surface and its interior for effective thermoregulation. In *E. lanestris* the tightly woven tent becomes enlarged throughout larval development and serves as an essential requisite for behavioral and metabolic thermoregulation (Ruf & Fiedler 2000, 2002b) as well as shelter. In *E. catax*, however, the far smaller tent is rather constructed like a platform on which caterpillars rest and is situated much deeper within the bush. This position of the tent does not necessarily affect thermoregulation because blackthorns are still bare during the early development of the caterpillars and solar radiation easily reaches the larvae. During this early developmental stage caterpillars first feed on the blossoms and the emerging leaf buds and later on the young leaves (M. Dolek, pers. obs.). Due to the high nutrient content of blossoms and buds (Slansky 1993) caterpillars are able to complete the social phase of their development (i.e. from hatching until the beginning of the fourth instar) in less than 10 days under field conditions (A. Freese, unpubl. data). Thus, caterpillars normally have already abandoned the tent before the leaves expand to their final size and would therefore shade the tent. Therefore, it is

reasonable to suppose that doing without a large tent is a strategy selected for to save time and resources. Silk is an energetically costly investment for any insect (Berenbaum et al. 1993; Craig et al. 1999, Stevens et al. 1999) and spinning silk also requires time. Thus, a flat resting platform is sufficient for the caterpillars to assemble in a dense, flat aggregation which facilitates the absorption of the incoming solar radiation and provides the caterpillars with a boundary layer suitable for thermoregulation (Joos et al. 1988). Yet, the small platform-type tent of *E. catax* does not allow to be used as a retreat in times of inclement weather or under attack. In contrast to the statements of Bolz (1998) we never observed larvae of *E. catax* inside the tent. One might speculate that due to the early timing and high developmental speed *E. catax* larvae minimize the risk of predation and parasitism but comparative field studies on the main causes of larval mortality have not been conducted (for *E. lanestris* see Ruf & Fiedler, submitted).

In contrast to the two *Eriogaster* species *M. neustria* caterpillars did not stay at one single place during the social phase of their lives but usually built a successive series of tents or several parallel tents when groups subdivide. These observations are consistent with those made by Tutt (1900). Obviously, the tent is used as a communication center which enables the caterpillars to aggregate closely and possibly to deter predators by their pronounced head jerking behavior. Nevertheless, the tent is abandoned in order to get closer to new food findings.

Possibly, thermoregulative advantages which require more synchronized resting phases and a large tent are of minor importance in *M. neustria*. This assumption is supported by the light coloration (blue, white and orange stripes) of the caterpillars which is expected to be less suitable for thermoregulation than the black ground color of the two *Eriogaster* species. The more extensive geographical distribution of *M. neustria* as well as the wider range of habitats used also suggest that this species is less dependent on high insolation and effective thermoregulation than *E. lanestris* and *E. catax*.

Evolution of sociality in the genera Eriogaster and Malacosoma

Within the Lepidoptera sociality is a rare phenomenon but is widespread and has undoubtedly evolved multiple times (Costa & Pierce 1997). Factors promoting the evolution of sociality have been identified in several studies on moths and butterflies. Among these are the reduction of the proboscis which often leads to egg clustering for energetic reasons (Miller 1996, Tammaru & Haukioja 1996, Hebert 1983). Accordingly, gregarious larvae are more frequent in species that do not feed as adults (Hunter 1991). Furthermore, larval aposematism favors the evolution of gregariousness (Sillén-Tullberg 1988). Although the Lasiocampidae are of only minor diversity (ca. 1500 species in 150 genera, Lemaire & Minet 1999), larval sociality seems to be relatively frequent. Besides the genera *Eriogaster* and *Malacosoma* social CPF caterpillars are known from *Gloveria* and *Eutachyptera* (Franclemont 1973), and many other genera reportedly have gregarious caterpillars (e.g. Aurivillus 1930, Austara et al. 1971, Taylor 1949, Arce de Hamity & Neder de Roman 1981, Holloway 1987, Murphy 1990, Vuattoux 1991, Aherkar et al. 1997, Costa & Pierce 1997). Overall, gregarious caterpillars occur in more than 10% (18 out of 150) of the lasiocampid genera.

In all species of the genera *Eriogaster* and *Malacosoma* caterpillars are social (Table 8). In *Malacosoma* there is a continuum from species which build a permanent large tent, over species with small or multiple tents, to those species that do not build a tent at all (Table 8). In contrast, all species of *Eriogaster* for which literature data are available seem to build a tent with the possible exception of *E. rimicola* for which statements in literature are contradictory. It must, however, be emphasized that larval life-histories remain unknown for ca. 50% of the species within both genera. Moreover, available information is frequently incomplete, stems from old records and lacks any rigor in the way it has been recorded. Thus, more detailed accounts like the present one are required in order to gain more comparative insight into the evolutionary ecology of larval sociality in *Eriogaster* and *Malacosoma*.

Table 8. Foraging strategy and use of tent of six species of *Eriogaster*[1] and eleven species of *Malacosoma*[2]. CP central-place foraging, NOM nomadic foraging, CP/NOM multiple nests, frequently abandoned. Since most studies cited are only descriptive papers it was impossible to find all parameters for every species. 'small', 'large', 'dense', and 'loose' are necessarily arbitrary terms. Literature on *E. rimicola* is controversial.

Species	Foraging strategy	Tent	Reference
Eriogaster[1] *amygdali*	CP	Accessible interior	Talhouk 1975
Eriogaster arbusculae	CP	Small, dense, accessible interior	Trawöger 1977
Eriogaster catax	CP	Small, dense, surface only	This study
Eriogaster lanestris	CP	Large, dense, accessible interior	Ruf & Fiedler 2002b
Eriogaster philippsi	CP	Accessible interior	Talhouk 1940
Eriogaster rimicola	NOM? CP?	No tent or: tent with accessible interior	Lunak 1937, Gómez Bustillo & Fernández-Rubio 1976, Bertaccini et al. 1994
Malacosoma[2] *alpicolum*	CP/NOM	Loose, accessible interior	ProNatura 2000, Martín & Serrano 1984
Malacosoma americanum	CP	Large, dense, accessible interior	Fitzgerald 1995
Malacosoma californicum	CP	Large, accessible interior	Myers 2000, Stehr & Cook 1968
Malacosoma castrensis	CP/NOM	Loose	Martín & Serrano 1984
Malacosoma constrictum	CP/NOM	Series of small tents for molting	Stehr & Cook 1968
Malacosoma disstria	NOM	No tent	Fitzgerald 1995
Malacosoma franconcium[3]	CP/NOM	Loose, accessible interior	ProNatura 2000
Malacosoma incurvum	CP	Large tent, accessible interior	Filip & Dirzo 1985, Stehr & Cook 1968
Malacosoma indica	CP	?	Joshi & Agarwal 1979
Malacosoma neustria	CP/(NOM)	Small, dense, preferably on surface	Tutt 1900, this study
Malacosoma tigris	CP/NOM	Series of small tents for molting	Stehr & Cook 1968

[1] altogether there are about 13 *Eriogaster* species known so far (see: de Freina 1999, Dubatolov & Zolotuhin 1992).

[2] altogether there are about 25 species of *Malacosoma* known so far (Fitzgerald 1995).

[3] probably only a subspecies of *M. alpicolum.*

Tent building appears to be restricted to four genera within the Lasiocampidae, which do not share a last ancestor common only to them (Regier et al. 2000). Hence, together with outgroup data this suggests tent-building to be a derived character state that has multiply evolved in the Lasiocampidae. Since there is no reliable phylogenetic system of the two genera, it is presently impossible to decide if the small size of tents or their complete absence in some species is an ancestral or a secondary trait. For example, small tents used as mere resting platforms as described here for *E. catax* may represent an ancestral trait or may be the result of secondary reduction. A precise resolution of the phylogeny of these two genera will be required to gain more insight into the history and dynamics of the evolution of sociality in the Lasiocampidae.

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Chapter 12

Implications of the gregarious life-style on the development of *Araschnia levana*

Abstract

Females of the European Map butterfly, *Araschnia levana*, clump their eggs in clusters on the underside of leaves of their host plant, the stinging nettle, *Urtica dioica*. Resulting larvae feed gregariously during the first three instars. Aggregations break up with the beginning of the fourth instar and last instar larvae feed solitarily.

Group cohesion is achieved by trail marking and trail following behavior.

The hypothesis that aggregation increases growth rate was tested by rearing caterpillars in different group sizes (1, 10, 40, or 80 individuals, respectively) either on excised nettle leaves in plastic boxes in the laboratory, or on intact plants in a greenhouse, respectively.

In both experiments, grouped caterpillars showed a shorter developmental time and a higher growth rate compared to solitary caterpillars. However, differences were less pronounced in the greenhouse experiment which is attributed to higher ambient temperatures. Pupal weight tended to be relatively higher for grouped caterpillars in the greenhouse experiment but lower in the laboratory experiment. The latter effect is ascribed to crowding effects in the rearing containers during the later instars.

Mechanisms underlying faster development are discussed. It is supposed that collective overwhelming of host-plant's spines is most important for the observed effects.

Introduction

Although many moths are known to lay their eggs in large clusters, this phenomenon is relatively scarce in butterflies (Stamp 1980, Costa & Pierce 1997). Within butterflies, egg clustering is most common in the family Nymphalidae and resulting larvae often stay together for at least the initial instars.

In the Central European fauna gregarious nymphalid caterpillars can be found in most checkerspot butterflies (tribe Melitaeini, genera *Euphydryas*, *Melitaea*; e.g., Wahlberg 1995, 1997, Klemetti & Wahlberg 1997) and in many (though by far not all) Nymphalini butterflies (Ebert & Rennwald 1993). Among the latter belongs the European map butterfly, *Araschnia levana*. Females of this species lay their eggs in clusters consisting of a group of egg turrets on the underside of its only host plant, the stinging nettle (cf. Chapter 2). Caterpillars stay together until the beginning of the 4th instar, when colonies disintegrate and last instar caterpillars live and pupate solitarily.

There are many hypotheses to explain the evolution and maintenance of egg clustering and larval gregariousness in the Lepidoptera. Most studies concentrate on the protection of aggregated larvae from natural enemies (reviewed by Hunter 2000). I will deal with this subject in Chapter 13 as well. There are, however, other hypotheses that explain egg clustering and larval aggregations. Among these are energy limitations on adult females (Hebert 1983), prevention of egg desiccation (Clark & Faeth 1998) and increase in female fecundity (Courtney 1984). At least the first two factors are not likely to promote egg clustering in *A. levana* since females possess a functional proboscis and are observed regularly feeding on nectar plants. Furthermore, map butterflies prefer nettles in shaded and humid habitats for egg deposition (Ebert & Rennwald 1993), which rendering egg desiccation very unlikely. Some further studies concentrated on the enhancement of feeding efficiency in gregarious larvae, leading to faster development of grouped caterpillars. Two mechanisms were supposed to influence communal feeding: (1) advantages of caterpillar groups in overwhelming plant defensive structures (Young & Moffet

1979, Clark & Faeth 1997, Denno & Benrey 1997) and (2) increased social stimulation to feed (Long 1955).

Stinging nettles are densely covered with spines. These sharp spines contain histamine and formic acid, irritating chemicals that are released when the plant is touched (Rodriguez et al. 1984). Especially on young leaves these spines form a dense cover which complicates movements of small insects. This particularly holds for young caterpillars that have only short legs and are severely constrained in moving on such surfaces (C. Ruf, pers. obs.). Thus, caterpillars feeding on nettles can be expected to benefit from communally overcoming spines, for example by collective spinning of silk.

In this study I focused on developmental benefits of gregariousness in *A. levana*. I hypothesized that if gregariousness in *A. levana* is driven by the necessity of caterpillars to overwhelm plant defensive structures like spines, caterpillars that are reared in groups should develop faster and gain more weight than caterpillars reared in isolation. Within the grouped caterpillars I expected a decrease of developmental time and an increase of growth rate from small to large groups (Fig. 1). Relative alteration of pupal weight was either expected to equal growth rate or to firstly increase to a maximum at medium group sizes and decline at large group sizes if effects of food competition influence the development of the caterpillars (Fig. 1).

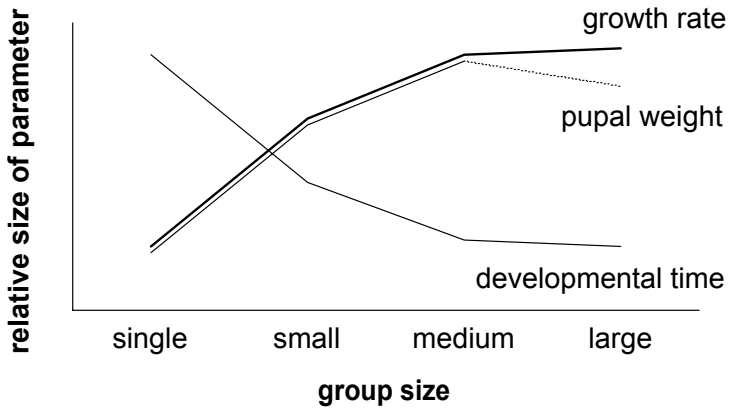

Fig. 1 – Hypothesized patterns of developmental parameters according to group size in *A. levana*, based on the assumption that gregariousness increases feeding efficiency and consequently development.

Decoupling of growth rate and / or pupal weight and developmental time has been shown frequently (Abrams et al. 1996, Nylin & Gotthard 1998). Therefore, I analyzed these three parameters separately as well.

The study followed three steps. (1) Appropriate experimental group sizes were determined by assessing the variability of natural egg clutch size. (2) Caterpillars were reared in plastic boxes on excised nettle shoots. Food was always in close proximity and it was easy to determine the weight of caterpillars. (3) Caterpillars were reared on potted nettle plants. This approach was more natural but it was impossible to remove and re-integrate caterpillars for weighing since disturbance may have prompted the caterpillars to drop off the plant. In an additional experiment I tested if caterpillars marked and followed trails that may ensure group cohesion.

Material and Methods

Breeding: Group size experiments were conducted using the progeny of wild caught females. For egg clutch size counts females of the first laboratory bred generation were used since females caught in the field are expected to have already laid parts of their egg load and resulting egg clusters may thus be smaller than on average.

Females of *Araschnia levana* (summer generation) were collected in the field in the vicinity of Bayreuth and transferred to plastic boxes (Volume: 10l). The bottom of each box was covered with cellulose tissue which was moistened regularly. The butterflies were provided with shoots or potted individuals of their host-plant (stinging nettle, *Urtica dioica*) for egg deposition. Each box was supplied with a small bunch of nectar plants (as available from the field), predominantly Asteraceae. Boxes were also provided with petri dishes with wet filter paper to make water available to the animals. Egg turrets were removed two days after deposition. Since females were never observed to oviposit on a leaf where another female had already laid eggs it is very unlikely that large clusters consisted of eggs from two females. Eggs were stored until hatching of the caterpillars in weighing dishes which were put into bigger boxes with

moistened tissue for optimal humidity. Resulting larvae were reared in plastic boxes (see below, experiment 1). Pupae were removed from the leaves or stems and glued on small paper strips (ca. 1 x 10cm), one beside the other (glue: Ponal® Express, Henkel KgaA, Düsseldorf). Paper strips were then hung up in plastic boxes (volume: 1000cm³) lined with wet cellulose tissue, so that the pupae hung upside down for eclosion. 5-10 males and a similar number of females were transferred to gauze cages (ca. 30 x 30 x 30cm) at 25°C (L:D 18:6h) for mating. Cages were equipped like the plastic boxes described above for egg-laying. Females were marked with a permanent marker (Lumocolor 313, Staedtler) during copulation and were then transferred to new plastic boxes for egg deposition. The number of eggs from 50 egg clusters of ca. 20 females was counted and eggs were treated as described above.

Fixing of experimental group size: Based on the number of eggs in a cluster, experimental group sizes were determined. 'Large' groups approximately refer to the maximum clutch size, 'medium' groups to mean clutch size and 'small' groups to minimum group size.

Group size manipulation: In order to create groups with different numbers of individuals, freshly hatched caterpillars were cautiously handled with a fine brush and transferred onto leaves of nettle. The numbers of caterpillars used for the two experiments described below are listed in Table 1.

Experiment 1: Caterpillars were reared in 250cm² plastic boxes outlined with moistened cellulose tissue and with fresh nettle leaves available *ad libitum*. Leaves were replaced every day to optimize food quality. After the third instar it was necessary to subdivide the individuals from medium or large groups respectively to more boxes (maximum: 20 individuals per box). Boxes were put into an environmental cabinet at 22°C with a 12:12h (L:D) light regime. The following parameters were recorded: Total duration of development, weight of pupae, and weight of third instar caterpillars two days after molting to L3. Body weights were measured to the nearest 0.1mg with an electronic balance (Sartorius MC 210 P). Sample size of L3 caterpillars was different and depended on group size (number of individuals (ind.) weighed per approach:

3 ind. / small groups, 5 ind. / medium groups, 10 ind. / large groups, all solitary caterpillars). Growth rate was calculated according to Gotthard et al. (1994). To determine the hatchling weight of caterpillars, 20 freshly eclosed caterpillars were pooled and weighed on an electronic balance to the nearest 0.01mg (Sartorius MC 210 P). Mean weight of these caterpillars (m=0.11mg) was then used for the growth rate calculations.

Experiment 2: Caterpillars were placed on potted nettles. It was controlled that all groups started on leaves of approximately equal size and age. Therefore I chose the third youngest pair of leaves of each stem and placed the larvae randomly on one of these leaves. All plants were comparable in size (height: ca. 30cm at the beginning of the experiments) and provided with the same volume of soil (pot diameter ca. 25cm) independently of group size to level out differences in food quality. All larvae crawled to the underside of the leaves within one hour after they had been placed on the leaf. Plants were placed in a non-heated greenhouse in August. Caterpillars were removed from the plants 3 days after molting to the fifth instar to prevent losses due to dispersion before pupation. Caterpillars were then reared in plastic boxes (Volume: 1000cm³) as described above until pupation.

Air temperature and relative humidity in the greenhouse was registered by a data logger (HandyLog DK 503 combined with software Infralog for Windows, both: Driesen + Kern GmbH, Bad Bramstedt).

Table 1. Number of replicate groups used in the two experiments.

		Group size			
		Single	Small (N=10)	Medium (N=40)	Large (N=80)
Number of larvae on	excised nettle leaves	29	25	15	5
	intact nettle plants	21	9	10	6

Trail following behavior: In order to test whether caterpillars of *Araschnia levana* mark trails and recognize marked trails, when they crawl over newly explored parts of a plant, I used a simple Y-test. Small parts of filter paper (3 x 1cm) were

folded along the long axis forming roof-like pieces. Three of these pieces were arranged as a Y (all angles 180°). Ten caterpillars were allowed to pass over these pieces, when one arm of the Y was removed. Afterwards, the second, yet unmarked arm of the Y was added. Test caterpillars (third instar) were then put on the stem of the Y and were allowed to crawl to the crossing point and decide for the marked or unmarked trail. 20 caterpillars (third instar) were tested. The same stem of the Y was used for all experiments. The side of the marked or unmarked arm was regularly changed after every test caterpillar to rule out side preferences.

Statistics: In order to compare the parameters between different group sizes I used medians of each group instead of original data to avoid effects of too large differences in sample size and pseudoreplication. For normally distributed data I used one-way analysis of variance. Data were squareroot-transformed for achieving normal distribution and / or homogeneity of variances if necessary and possible. Otherwise I used Kruskal-Wallis ANOVA for non-parametric data. Post-hoc tests used were Tukey's HSD test after standard ANOVA or the Tukey-Kramer test for non-parametric data.

In some parameters tested there was a marked difference in variances between different group sizes, especially in weight measurements between solitary and grouped caterpillars. To test if these differences were caused by the use of medians in groups of 10 to 80 individuals that reduced variances or if these differences indicated real variability between the groups I calculated coefficients of variation which were then tested for significance (following Sokal & Braumann 1980, see formulas below).

Coefficient of variation: $V^* = \left(1 + \frac{1}{4N}\right) \times \frac{s}{\bar{x}}$

Standard error of V:* $\sigma = \sqrt{\left[\frac{V^{*2}}{2N}\left(\frac{N}{N-1} + 2V^{*2}\right)\left(1 + \frac{1}{4N}\right)^2\right]}$

Calculation of t: $t = \frac{V^*_1 - V^*_2}{\sqrt{\sigma_1^2 + \sigma_2^2}}$

where *s* = sample standard deviation,

$\bar{x}$ = sample mean,

N = sample size

t-Tests were calculated pairwise and resulting p-values were Bonferroni corrected (sequential Bonferroni procedure, Hochberg 1988) to assess the significance of differences.

Results

Size of egg clutches

The number of eggs per clutch varied over a wide range, but reasonably followed a normal distribution. One egg cluster may consist of one to six egg turrets. The average number of eggs in a cluster was 42 with a maximum of 75 eggs and a minimum of 7 eggs (Fig. 2). This distribution led to the fixing of experimental group sizes at 10 (small group), 40 (mean = medium group size) and 80 individuals (maximum = large group size).

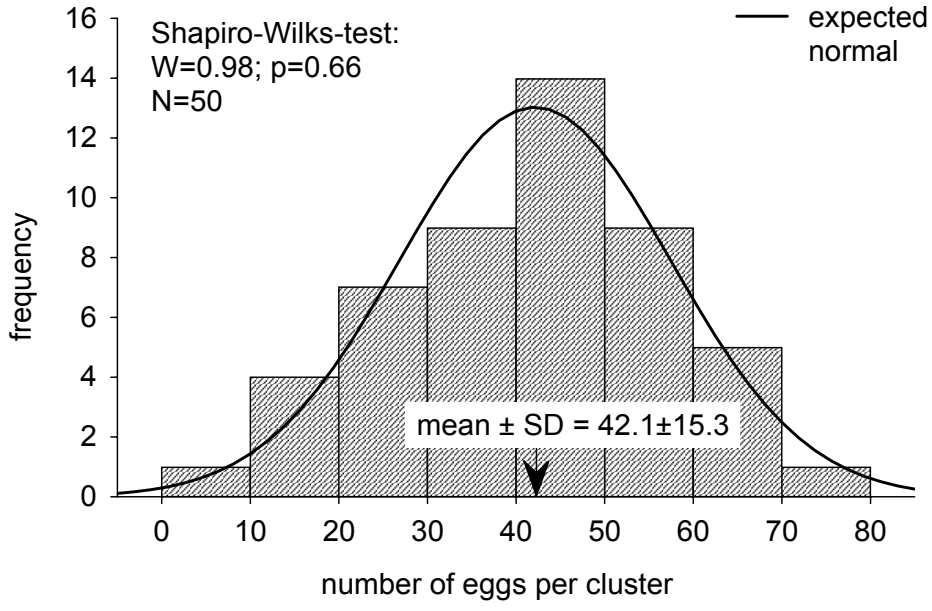

Fig. 2 – Variability of the number of eggs laid per cluster by *Araschnia levana*. Data from 50 clusters laid by females of the first laboratory bred generation (non-diapause progeny of females of the summer generation caught in the field).

Development on excised nettle leaves

Caterpillars in groups, irrespective of group size, had a significantly shorter developmental time than caterpillars which were reared individually. This difference between single and grouped caterpillars was already visible in 3rd instar caterpillars that were bred in groups of 40 or 80 individuals and became very obvious at the end of the larval period (Fig. 3, upper graphs). Solitary larvae lagged behind for 2-3 days.

Body mass of 3rd instar caterpillars was highest in groups of 40 or 80 caterpillars but was significantly lower in groups of 10 individuals and again significantly lower in singly bred caterpillars (Fig. 3: middle). Interestingly, this effect was reversed regarding pupal mass: caterpillars reared in large groups produced the lightest pupae.

Growth rate proved to be significantly lower in singly bred caterpillars compared to grouped caterpillars irrespective of group size during the social phase of the caterpillars' development. In contrast, growth rate was indistinguishable between all group sizes during the last two (i.e. solitary) instars in all experimental groups (Fig. 3, lower graphs).

Development on intact plants

Caterpillars on potted nettle plants reared in groups of 40 individuals developed significantly faster and showed a higher growth rate than singly bred caterpillars. No other differences between groups could be ascertained statistically (Fig. 4). Mean pupal weight was 6-8% lower in singly bred caterpillars compared to medians of grouped caterpillars but differences were not significant. Obviously, caterpillars reached their physiological limits under the hot weather conditions prevalent during the study phase (cf. Fig. 5). Due to high outside temperatures the greenhouse heated to above 30°C and ambient air temperature even exceeded 40°C on three days.

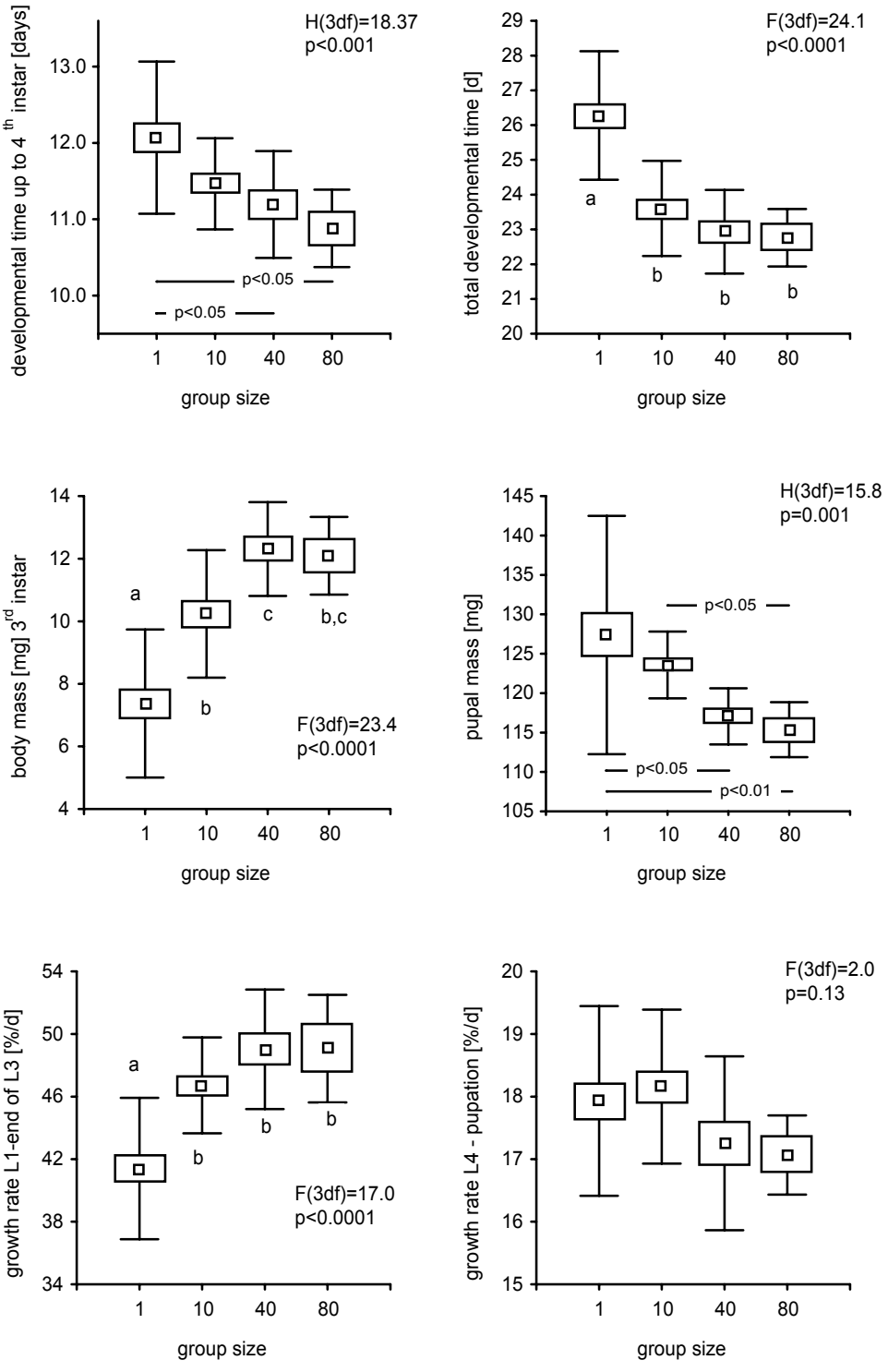

Fig. 3 – Developmental time, weight and growth rate of *A. levana* caterpillars fed with excised nettle shoots in closed rearing containers. Left column: Data from first three instars, right column: data from total development, last two instars, or pupae. Box-Whisker plots show mean, ±1SD and ±1SE. Boxes marked with different letters differ significantly.

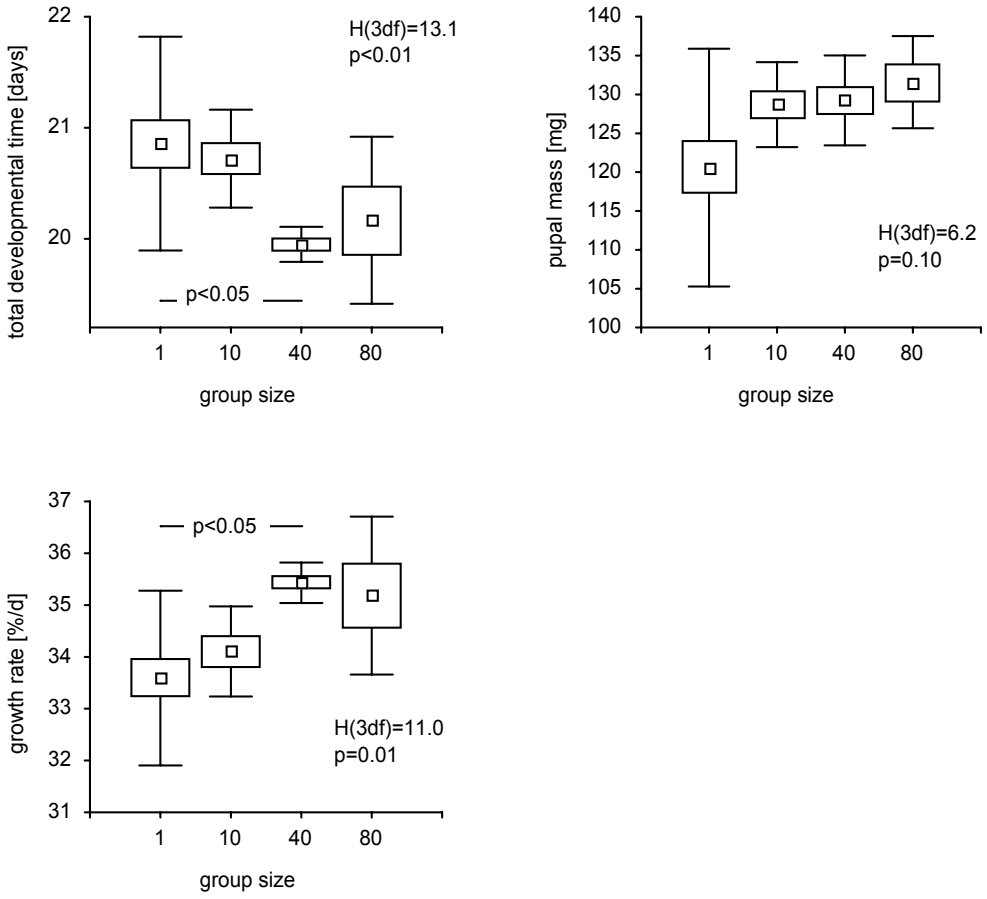

Fig. 4 – Developmental time, pupal mass and growth rate of *A. levana* caterpillars reared on potted nettle plants in the greenhouse. Box-Whisker plots show mean, ±1SD and ±1SE.

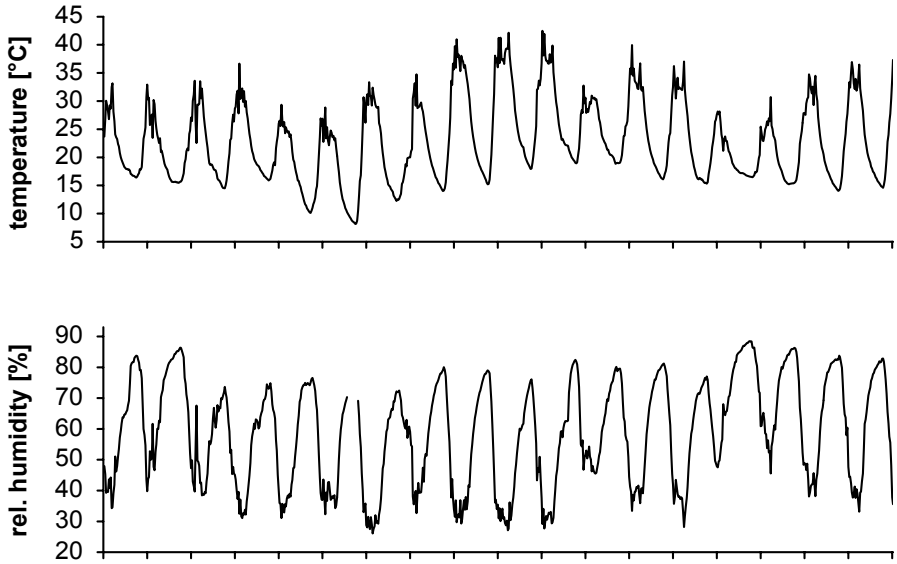

Fig. 5 – Course of air temperature and relative humidity in the greenhouse during the rearing experiment (from L1 until collecting L5 larvae off the plant). Each space between two ticks on the x-axis corresponds to one day (7th – 24th August). Mean temperature: 23°C, maximum 42.5°C.

Variability of parameters in different group sizes

There was a large inhomogeneity of variances in all three parameters tested in the greenhouse experiment and in pupal mass data in the plastic box experiment. Table 2 shows coefficients of variation for these data.

Table 2. Coefficients of variation calculated (1) from the median data for grouped caterpillars or (2) from the original data (with all individuals of each group pooled). G = greenhouse experiment, PB = caterpillars reared in plastic boxes.

Group size	Calculated from medians				Calculated from original data			
	1	10	40	80	1	10	40	80
Pupal mass G	0.128	0.044	0.046	0.047	0.128	0.090	0.103	0.110
Develop. time G	0.047	0.022	0.008	0.039	0.047	0.036	0.031	0.041
Growth rate G	0.051	0.026	0.011	0.045	0.051	0.038	0.034	0.041
Pupal mass PB	0.120	0.042	0.035	0.038	0.120	0.097	0.110	0.121

Pairwise comparisons of coefficients of variation showed that variances were often significantly larger in singly bred caterpillars compared to the data of

grouped caterpillars if median data were used (Table 3). However, these significances broke down if original data were used. In this case, large groups sometimes differed significantly, probably an effect of large sample sizes. Thus, large differences in variability are an effect of the use of group medians and are not caused by a real higher variability of the parameters in singly bred larvae.

Table 3. Significant differences between coefficients of variation (see Table 2) (Sokal & Braumann 1980). Double arrows indicate significant differences, numbers in parentheses indicate group sizes. G = greenhouse experiment, PB = caterpillars reared in plastic boxes.

	Calculated from group medians	Calculated from original data
Pupal mass G	(1) ↔(10, 40, 80)	-
Developmental time G	(1) ↔(10, 40)	(40) ↔(80)
Growth rate G	(1) ↔(40)	(40) ↔(80)
Pupal mass PB	(1) ↔(10, 40, 80)	(10) ↔(80)

Trail following experiment

All 20 caterpillars tested preferred the marked arm of the Y to the unmarked trail (χ^2(1df)=20.0; p<0.0001). The stem section of the Y was always connected to the marked arm of the Y by fine filaments of silk.

Discussion

Development and growth rate

Experiments with *A. levana* caterpillars feeding either on excised nettle shoots or on intact plants showed that there is a continuous decrease of development time and a continuous increase of growth rate with group size. However, in the majority of cases these tendencies could not be partitioned statistically between grouped caterpillars but only between grouped versus solitarily bred caterpillars. Obviously, caterpillars benefit from their gregarious life-style even in small groups and further developmental benefits in larger groups are small. Many other studies have shown that larger groups of gregarious caterpillars have shorter developmental times and higher growth rates compared to smaller

groups (Long 1955, Tsubaki 1981, Tsubaki & Shiotsu 1982, Lawrence 1990, Clark & Faeth 1997, Denno & Benrey 1997).

Mass effects

According to the expectations caterpillars reared in groups in the greenhouse tended to produce larger pupae. This effect has also been shown for other gregarious caterpillars (Tsubaki 1981, Fordyce & Agrawal 2001, Fitzgerald & Visscher 1996). In the greenhouse experiment, colonies had the possibility to disintegrate in the fourth instar and plants were large enough to allow for a wide dispersion of the individuals.

Contradictory to the result discussed above, pupal mass was lowest in grouped caterpillars in the laboratory experiment, although the body mass of third instar caterpillars was highest for larger groups at first. This effect is probably caused by developmental stress of grouped caterpillars in the last two instars that would not live gregariously under field conditions beyond the third instar. This assumption is also supported by the fact that growth rate within the first three instars is significantly higher for grouped caterpillars (i.e. caterpillars benefit from the presence of their siblings) whereas there is no difference between grouped and single caterpillars for the last two larval stadia. Obviously, the rearing conditions were suitable for young caterpillars but caused environmental stress for late instar caterpillars. Effects of food shortage can be excluded since caterpillars were always fed *ad libitum* but tactile contacts between the caterpillars in the rearing boxes (1000cm³) were probably very frequent. In *Hyphantria cunea*, for example, Suzuki (1981) found a sharp decrease of pupal weight and female fecundity with increasing group size in a laboratory experiment which was attributed to overcrowding effects in the later instars.

Trail following behavior

Caterpillars of *A. levana* prefer trails marked by their siblings to unmarked trails. Trail marking ensures the cohesion of group members while crawling to a new foraging site without having direct body contact. Similar trail following behavior has also been shown for the nymphalid *Chlosyne lacinia* (Bush 1969). I did not test explicitly whether there is a trail marker eliciting trail following behavior, or if

the small silk trails alone serve this purpose. Other studies on nomadic or patch-restricted foragers (terms: see Chapter 1) suggest that the latter is the predominant mode of trail communication in these species (e.g. Fitzgerald 1993). The existence of a trail based communication in *A. levana* stresses the importance of group cohesion, i.e. these caterpillars are not only mere aggregates of individuals feeding on the same parts of the plant but actively hold contact to their siblings. For a general discussion of trail-based communication see Chapters 7+8.

Mechanisms of developmental advantages of gregarious caterpillars

Many plants employ mechanical defenses and insect herbivores have developed a number of strategies to overcome these defenses (Dussourd 1993 and references therein). Trichomes have been shown to act as structural defense (reviewed in: Southwood 1986). For example, caterpillars of *Pieris brassicae* cause higher damage on *Brassica* plants that had been selected for low trichome density (Ågren & Schemske 1993). Furthermore, Colorado potato beetles preferentially feed on leaves where trichomes were removed (Yencho & Tingey 1994).

As described above, developmental patterns were quite similar in the two experimental approaches used in this study. However, the effect of group size was stronger in the laboratory experiment, although food was more easily available to the caterpillars since they were not forced to pass the hairy leaf stalks and stems of the plants. Probably, the weakness of effects in the greenhouse experiment must be attributed to the high temperatures prevalent during the study. Obviously, caterpillars reached their physiological maximum with regard to growth at regular daytime temperatures above 30°C (cf. Slansky 1993 and references therein) and in the absence of sufficient scope for plastic responses effects of group size were thus very weak.

Enhanced growth rates have also been attributed to social feeding stimulation. It has been argued that aggregations may lead to an increase in overall activity among crowded larvae (e.g. Long 1953, 1955). However, it is most unlikely to assume that the presence of a caterpillar's siblings should be primarily

necessary to enhance a caterpillar's internal feeding rhythm. Thus, I assume that collective feeding stimulation – if it does occur at all – is a consequence of larval gregariousness rather than a factor promoting the evolution of sociality in caterpillars. Studies on eastern tent caterpillars (*Malacosoma americanum*) showed that group living appears to constrain rather than promote foraging activity (Fitzgerald & Visscher 1996). Isolated caterpillars of this species foraged significantly more often than caterpillars maintained in groups but grew more slowly compared to grouped caterpillars. It was hypothesized that the slower growth may reflect the increased metabolic costs associated with increased activity or restricted food intake.

Although feeding stimulation might play a role in the enhanced development of grouped caterpillars in *A. levana*, it is more likely to suppose that grouped caterpillars are better able to overwhelm the nettle's spines efficiently by spinning silk. It is generally acknowledged that silk enhances the adherence of caterpillars to the leaf (Dussourd 1993 and references therein). Furthermore, caterpillars of *A. levana* feed in tight aggregations synchronously at the edges of the nettle leaves (cf. Chapter 2, plate 4). It thus seems feasible that this behavior also serves inducing of a nutrient sink or overwhelming an induced allelochemical response in the plant (cf. Denno & Benrey 1997). Further experiments combining closer observations of the feeding behavior of *A. levana* with supplying artificial food without plant defensive structures (cf. Clark & Faeth 1997) are necessary to finally address this question.

Within the tribe Nymphalini there is an ancestral association with Urticaceae and many species still have the capacity to feed on *Urtica dioica* regardless of actual host-plant use (Janz et al. 2001). In Central European Nymphalini there are five species using the stinging nettle as their host-plant (Table 4). It is noticeable that three of these species have gregarious caterpillars. Another species (*V. atalanta*) lives solitarily but builds a leaf shelter in which it rests and feeds. The only polyphagous species (*P. c-album*) also builds a loose leaf shelter.

Table 4. Use of host-plants and life-style in nettle feeding, nymphalid butterfly larvae (all tribe Nymphalini) occurring in Central Europe. Data based on Janz et al. (2001)

Species	Host-plant families	Life-style
Araschnia levana	Urticaceae	gregarious
Aglais urticae	Urticaceae	gregarious
Inachis io	Urticaceae, Cannabidaceae	gregarious
Vanessa atalanta	Urticaceae	solitary, leaf shelter
Polygonia c-album	Urticaceae, Ulmaceae, Cannabidaceae, Salicaceae, Betulaceae, Grossulariaceae, Corylaceae	solitary, loose leaf shelter

Other species of Lepidoptera feeding on stinging nettle also frequently build leaf shelters, for example the noctuids *Diachrysia chrysitis* and *Hypena proboscidalis* (Carter & Hargreaves 1987, Porter 1997) and also some pyralid moths (e.g. *Pleuroptya ruralis*, *Eurrhypara hortulata*, *Udea olivalis*; Emmet 1979, Palm 1986). However, building leaf shelters is a common phenomenon among pyralid caterpillars (Slamka 1995). Nevertheless, it seems reasonable to suppose that caterpillars feeding on stinging nettles are severely constrained by the hairy leaves and stems and follow either of two strategies: (1) Caterpillars feed solitarily. In this case they preferentially feed within leaf shelters were they may feed slowly while they are hidden. (2) Caterpillars feed more openly but live gregariously and benefit from communal feeding facilitation and may develop faster.

However, there are numerous Nymphalini butterfly species that have gregarious caterpillars but use other host-plants that do not possess hairy leaves. Among these are the polyphagous tree-feeding *Nymphalis antiopa*, *Nymphalis milberti*, *Nymphalis vau-album*, *Nymphalis xanthomelas*, and *Nymphalis polychloros* (Scott 1986, Ebert & Rennwald 1993, Costa & Pierce 1997). Thus, it seems more likely that gregariousness in Nymphalini butterflies is not bound to nettle feeding which is a phylogenetically old trait but is very plastic and has evolved numerous times (see cladistic analyses in Janz et al. 2001, Nylin et al. 2001). Nevertheless, host-plant architecture might have influenced the evolution and

the maintenance of gregariousness in *A. levana* but is surely not the main factor (see also discussion in Chapter 13).

Faster development in itself has of course no selective advantage but acts through mortality agents. Faster growth may result in less overall mortality for larvae feeding in larger groups (Clancy & Price 1987). Denno & Benrey (1997) demonstrated that slower growth rate extended the window of vulnerability to a wasp parasitoid, and thereby increased parasitism.

Thus, although the proximate mechanisms underlying faster development in *A. levana* need further investigations, developmental benefits are undoubtedly an important ultimate factor contributing to the gregarious behavior of the larvae of this species.

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Chapter 13

Group size influences larval survival of *Araschnia levana*

Abstract

Caterpillars of *A. levana* live together in aggregations of less than 10 up to over 80 individuals. Survival probability was independent of group size if caterpillars were reared on excised nettle leaves in plastic boxes (enemy free situation). In contrast, if caterpillars were reared on potted nettles in a greenhouse, where predators had free access, survival was highest for experimental groups of 40 individuals (=mean natural group size), medium for groups of 10 or 80 caterpillars, respectively, and very low for individual caterpillars.

If 30 caterpillars were exposed on potted nettles in the field, either as a tight group or spread over the plant, the survival rate of grouped caterpillars was significantly higher, indicating that different survival rates are not caused by a dilution effect (reduced per capita risk).

Caterpillars showed synchronized flicking behavior and strongly regurgitated foregut content when they were disturbed. Ants responded with marked defensive reactions when they were confronted with droplets of the regurgitated fluid. By synchronous flicking and regurgitating caterpillars were able to repel predatory bugs of equal body size. Caterpillars may only regurgitate once or twice in one sequence and are not always able to regurgitate large amounts of fluid, probably depending on their digestive condition. Thus, transient deficiencies of individual caterpillars in defensive capacity can be counterbalanced by grouping.

It is concluded that group defensive behavior is a key factor for the maintenance of gregarious behavior of larvae of this butterfly species.

Introduction

Assemblages of lepidopteran caterpillars vary broadly in size, ranging from only few individuals to aggregates containing hundreds or thousands of larvae. Variation in group size between species is largely influenced by various species-specific life-history traits and niche-associated constraints, but within species variation in colony size is also considerable (Fitzgerald 1993). Naturally, caterpillar groups consist of the offspring of one single female only, i.e. the members of the group are all siblings. Thus, the initial group size is totally determined by the female, unless high population densities or the lack of suitable host-plants lead to multi-family aggregates (e.g. Stamp 1981, Fitzgerald & Willer 1983).

The female herself is constrained by the total number of eggs available which in turn is influenced by larval nutrition (in semelparous insects, Tammaru & Haukioja 1996) and / or by adult feeding of the female (in iteroparous insects, e.g. Fischer & Fiedler 2001). Thus, in semelparous insects the clutch size is fixed at emergence and cannot be influenced by the female any more since all eggs are laid in one single cluster. In contrast, iteroparous insects that lay their eggs in a number of distinct groups are able to influence the success of their offspring by adjusting the number of eggs per cluster as well as the number of clutches to be laid.

The adjustment of egg clutch size is affected by extrinsic constraints acting on the females, but also by constraints relevant to the caterpillars. On the part of the females these constraints include, for example, the mortality risk between egg depositions, the time which is necessary for the maturation of new eggs, or the time required for the search for new, suitable host plants. For example, high mortality risks should lead to egg clutches that are larger than the clutch size that would be optimal if only success of the offspring were to be considered (Parker & Courtney 1984). On the part of the caterpillars constraints affecting group size are numerous, as well. Although group living may confer a variety of physiological, reproductive, and defensive benefits, it also potentially carries competitive and mortality costs (Vulinec 1990). For example, larval

aggregations may cause local food shortage, resulting in starvation or at least exposure to the risks of traveling to find fresh food. The issue, however, is clearly complex, and a complete explanation requires simultaneous consideration of many life-history characters.

Apart from the constraints influencing clutch size, it is most important for the females to maximize their individual fitness, i.e. to maximize the number of offspring surviving to the next generation. Thus, if the adjustment of egg clutch size is adaptive one should predict that survival of caterpillars is maximal in group sizes that equal the mean natural group size.

Survival rates should not only be influenced by the mere number of caterpillars in a cluster, but also by their ability to deter predators. Protection from natural enemies has been reported for many species of gregarious folivores and is prevalently acknowledged to be the most important benefit of gregariousness (Vulinec 1990, Hunter 2000). Mechanisms underlying this observations are quite complex and may interact with each other: Individuals in groups may benefit from increased chances of detecting predators, from active group defense against predators (Stamp 1981, Vulinec 1990, Codella & Raffa 1993), or from the dilution effect (as group size increases, per capita risk of attack decreases; Hamilton 1971, Turchin & Kareiva 1989).

This chapter aims at analyzing the effect of group size on the survival of gregarious caterpillars of the European Map butterfly, *Araschnia levana*. Females of this species lay several egg clusters on the leaf underside of their only host-plant, the stinging nettle, *Urtica dioica* (Ebert & Rennwald 1993). Preliminary studies have shown that clutch size varies enormously in *A. levana* (Chapter 12) but that study did not address the egg clutch size and its life-time variability within individual females. Moreover, nothing is presently known about survival rates of these caterpillars in different group sizes and defensive mechanisms have never been reported for this species.

Therefore, the following questions are addressed here:

1. Which parameters influence egg clutch size, i.e. are there any constraints on the part of the females?

2. Do caterpillars survive better in larger groups? Is there an optimal group size? What are the mechanisms underlying differential survival in different group sizes?
3. What behaviors do these larvae exhibit in response to being attacked? Are caterpillars at all able to defend themselves successfully against natural enemies?

Material and Methods

General breeding conditions: Females of *Araschnia levana* (summer generation) were collected in the field in the vicinity of Bayreuth and transferred to plastic boxes (Volume: 10l) in an environmental cabinet (L:D 18:6h, 22-25°C during the day / 15°C at night). The bottom of each box was covered with cellulose tissue which was wetted regularly. The butterflies were provided with stems or potted plants of stinging nettle (*Urtica dioica*) for egg deposition. Boxes were also provided with petri dishes with wet filter paper to make water available to the animals. Egg turrets were removed every second day and stored until hatching of the caterpillars in weighing dishes which were put into bigger boxes with moist tissue for raising humidity.

Breeding conditions for the 'female' experiment: Initial observations showed that the size of egg clusters varied with the age of the females. Therefore, I pursued studies on the egg laying dynamics of individual females. Butterflies of both sexes (ca. 50-100 individuals) were held together in a gauze cage (75 × 75 × 55cm) with big bunches of nectar plants at 20°C during seven hours of a day and 12°C during the rest of the day (L:D 12:12h). These restrictive conditions proved to be suitable for mating and survival of the butterflies that are otherwise quite short-lived. Due to the experimental conditions it was not possible to control exactly for the age of females prior to mating. Thus, females mated between the first and third day after eclosion from the pupa. Map Butterflies always mated during the evening hours and remained in copula for several hours. Females in copula were marked with a marker pen (Lumocolor 313, Staedtler) and were isolated on the following day before the onset of the warm

phase. They were individually transferred to a plastic box as described above. Nettles were checked very carefully for eggs clusters once a day during the cold phase. Egg clusters were removed, and eggs were counted and stored until hatching of the caterpillars. One egg cluster is defined here as the whole number of eggs laid by one female within one day, i.e. the sum of eggs of all egg turrets. All egg turrets were usually found tightly grouped on the same leaf underside.

Group size experiments: Caterpillars were cautiously handled with a fine brush and transferred onto leaves of nettle creating different group sizes or exposing single individuals, respectively. Three different survival experiments were conducted.

Experiment 1: Caterpillars (singly, and in groups of 10, 40, and 80) were reared in 250cm² plastic boxes with moistened cellulose tissue with excised nettle leaves available *ad libitum*. Leaves were replaced every day to optimize food quality. After the third instar it was necessary to subdivide the groups of 40 and 80 individuals respectively to more boxes (maximum: 20 individuals per box). Boxes were kept in an environmental cabinet at 22°C and a 12:12h (L:D) light regime. At the end of the caterpillars development praepupae and resulting pupae were counted to determine mortality rates. For sample sizes see Table 1.

Experiment 2: Caterpillars (singly, and in groups of 10, 40, and 80) were placed on potted nettles situated in a non-heated greenhouse (cf. Chapter 12). It was controlled that all groups started on the third youngest leaf of a stem and that all plants were comparable in size (height: ca. 30cm at the beginning of the experiment) and provided with the same volume of soil (diameter of pots: ca 25cm) independently of group size to minimize differences of food quality. All larvae crawled to the underside of the leaves within one hour after they had been placed on the plant. Invertebrate predators (such as small bugs, lacewing larvae, spiders, and harvestmen) had free access to the greenhouse and thrived there in abundance. Survival of the caterpillars and larval instar was recorded every day starting on the third day after their placement on the plant. For single caterpillars I placed small pieces of paper on the leaf where the caterpillar was seen during the last inspection to facilitate re-location on the next

day. Caterpillars were removed from the plants 3 days after molting to the fifth (last) instar to prevent losses due to dispersal off the host-plant before pupation. Caterpillars were then reared in plastic boxes as described above until pupation.

Experiment 3: In order to assess whether dilution effects might influence survival rates another experiment was conducted under field conditions. Thirty large pots (diameter: ca. 25cm) with nettles grown in a greenhouse (height: ca. 30cm) were placed along a small path crossing an arboretum in the Botanical Garden of the University of Bayreuth one week prior to the onset of the experiments. Thus, natural predators could settle down on the plants. Although *A. levana* butterflies are common in the Botanical Garden, egg laying by wild females should not have influenced our experiments since the flight period of the butterflies was almost over at the date when the nettles were put to their final position and caterpillars were released on the plants (7^{th} & 8^{th} August 2002). Since microclimate might have differed spatially (at various sites along the path) and temporally, the plants were positioned pairwise with each plant in a pair representing one of the two different treatments (see below). All caterpillars of all replicates were exposed within 1.5 days with the pairs being exposed with a maximum temporal difference of half an hour.

30 caterpillars that had just hatched were placed on each potted nettle. In half of the replicates ($N_{grouped}$=15) these caterpillars were grouped together on one leaf as a tight aggregate. In the other experiment ($N_{singles}$=15) the caterpillars were spread individually over the various shoots of the plants. Since all caterpillars had to be placed on the leaves during severe rainfalls they were settled directly on the underside of the leaves. It was paid attention that all caterpillars gripped the leaf before it was turned round so that no caterpillar fell off the plant. No matter if caterpillars were grouped or not, they were released at the third youngest leaf of a stem to provide equal nutritional starting conditions.

Grouped caterpillars were monitored once every day and the number of surviving individuals was counted. Monitoring of individually placed caterpillars was not possible due to their large number (N_{total}=450) and because of their

small size. At the end of the caterpillars' third instar all caterpillars were collected from the plants and were counted (on the 23rd August 2002, i.e. caterpillars were exposed to predation for 15-16 days). All plants were checked very carefully to avoid overlooking individual caterpillars.

Regurgitation experiment: Regurgitation behavior was observed with the help of a stereo-microscoope and documented by drawings and photographs.

Thirty bugs (*Nabis brevis,* Fam. Nabidae, ca. 7mm body length) were caught in the field (in the Botanical Garden) from nettle plants and starved for 5-7 days at room temperature. 5-10 individuals were then placed in a foraging arena (17 x 12cm plastic box with a 0.5cm thick plaster of Paris layer on the ground to provide sufficient humidity). The sides of the arena were prepared with a fluoropolymer coating (Dyneon™ TF 5035) to prevent the bugs from escaping. A group of ca. 30 caterpillars of *A. levana* (3rd instar, 6-8mm long) resting tightly together on a nettle leave was placed in the middle of the arena. Bugs were observed if their attacks were successful or not and were removed from the arena afterwards. Caterpillar groups were replaced if the first individuals started to crawl away. Altogether, 20 attacks were followed in detail.

Regurgitated fluid was gained from fourth or fifth instar caterpillars. The fluid was either removed by gently pressing a 20µl capillary onto the head of the caterpillars, or the caterpillars were cautiously held with a forceps right behind the head and the capillary was pressed at the mandibles to remove the regurgitated fluid.

For quantitative measurements the fluid of individual larvae (N=40, fourth instar) was taken up with a capillary, and the fluid level was measured with a digital caliper rule. Afterwards, the data were calibrated with the help of water droplets with a defined volume produced with the help of an Eppendorf pipette (vol. 100µl) to calculate the volume of the regurgitate droplets.

100µl of the regurgitated fluid and 100µl of water, respectively, were trickled at a distance of 3cm on a petri dish (diameter ca. 5cm) and offered to a colony of ants (*Lasius flavus*) in the proximity to a nest entrance. The reaction of 40 ants touching a droplet with their antennae was categorized as either 'defense

reaction' (ant immediately winces or bends its abdomen towards the droplet) or 'no reaction' (ant does not wince and leaves the droplet without any visible reaction) or 'drinking' (imbibing the liquid). The experiment was repeated with three different ant colonies.

Special statistical methods: Survival analysis: The Kaplan-Meier survival function estimation used in this study is a descriptive method for estimating the distribution of survival times from a sample (see details in StatSoft 1995). A comparison of the survival in more than two groups can be calculated. This multi-sample test computes a Chi-square value based on a score that is first assigned to each survival time using Mantel's permutation procedure (see details in StatSoft 1995). However, as implemented in the program version available (STATISTICA 5.5), this test is only able to compare up to 10 groups. Thus, for the field experiment analyses were calculated separately for caterpillar groups exposed on the 7th August or 8th August, respectively. Localization of significances was achieved by using pairwise Cox-Mantel tests with sequential Bonferroni correction (Hochberg 1988).

Montecarlo statistics on R×C matrices: For analyzing contingency tables of frequency data there is a restriction with regard to the cell entries. Thus, Chi-square analyses are not permitted if about 20% of the cells contain expected values <5 (Sachs 1992). In this study I therefore used a randomization method. For this analysis, 100.000 random matrices were generated with the same row and column totals as in the empirical matrix (see Blüthgen et al. 2000 and references therein). Statistics of the empirical data (T_{obs}) are then compared with the distribution of the statistics of all generated random matrices (T_{ran}) and the significance level p is calculated from the proportion of all T_{ran} being equal to or smaller than T_{obs}.

Results

Natural group sizes and female reproductive parameters

Egg cluster size varied between a minimum of 6 eggs and a maximum of 90 eggs (mean±SD=42.8±18.6, pooled data for all clutches of all females, N=87) (Fig. 1). The number of caterpillars hatching in each cluster was only slightly lower since only a small proportion of eggs was not viable (mean±SD=38.6±20.4). Overall, egg hatching rate was 86.6% under the experimental conditions. First clusters of an individual female were significantly larger, i.e. they contained nearly twice as many eggs compared to any subsequent cluster (Fig. 2). There was no significant decline of clutch sizes between second and subsequent egg clusters (Scheffé-test following ANOVA, $p>0.05$). Hatching rate declined from first clusters (95.5%), over second clusters (92.6%) to later clusters (81.8%), but differences were not significant due to large variances of hatching rate in late clusters (Kruskal-Wallis ANOVA: $H(2df)=1.25$, $p=0.3$). Nevertheless, hatching rate was negatively (but weakly) correlated with the age of the females ($r=-0.34$, $p<0.01$).

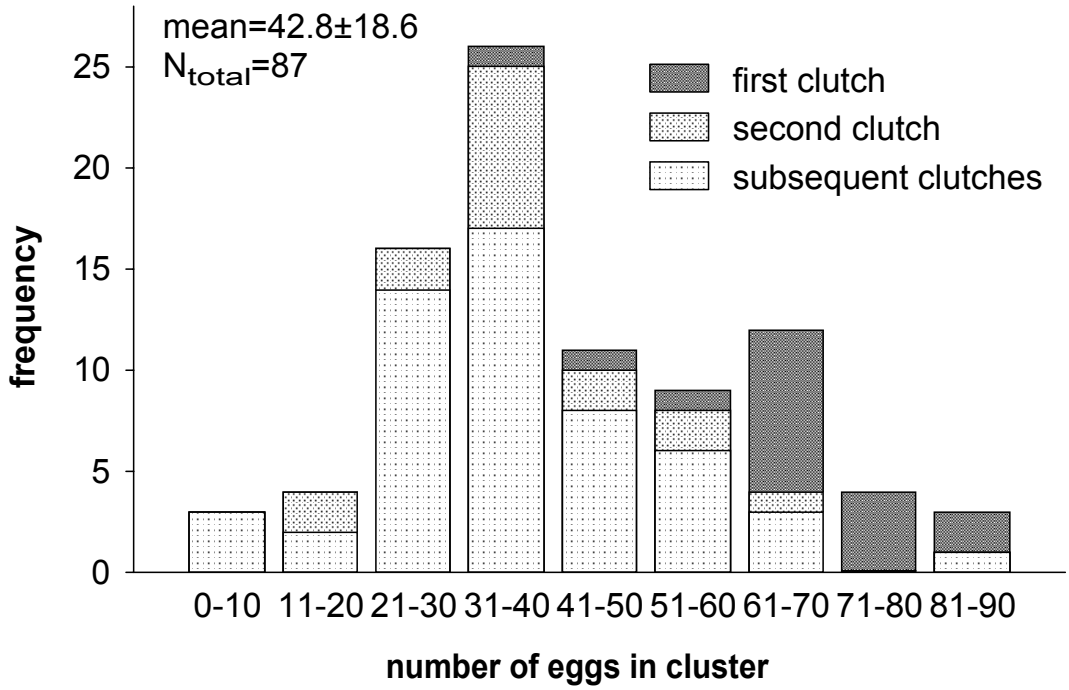

Fig. 1 – Frequency distribution of cluster size of *Araschnia levana*. Eggs laid under laboratory conditions. Pooled data from 17 females.

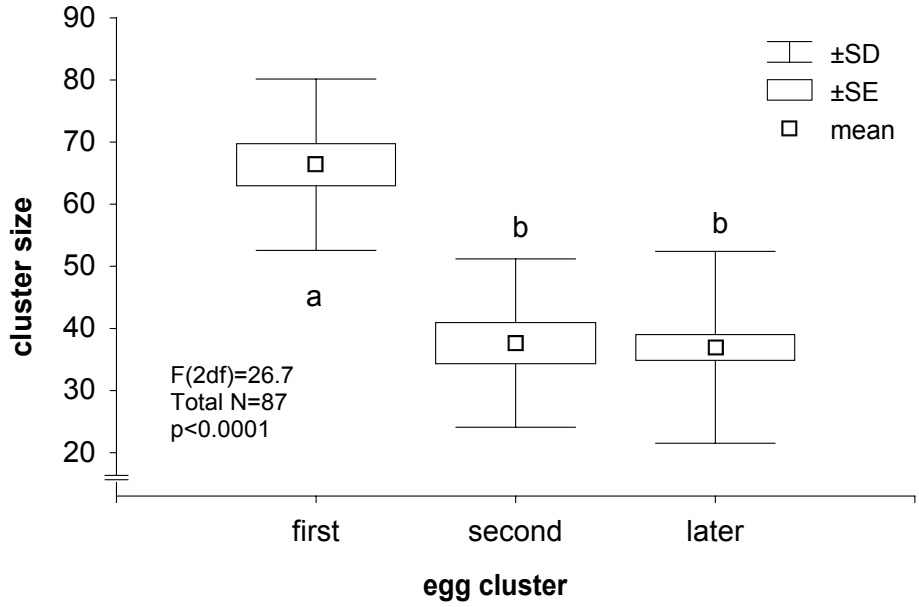

Fig. 2 – Size of egg clusters according to their succession in the female's life. Different letters indicate significant differences located with Scheffé's post-hoc test following ANOVA. Data were squareroot-transformed prior to statistical analysis to achieve normally distributed data.

Life time fecundity was quite variable and ranged between 71 and 298 eggs per female (mean±SD =219±80.3, N=17). Females laid an average of 5.2±2.0 egg clusters over their life time (min=2, max=9). Preoviposition period was short. Most females laid their first egg clutch on the day after mating. Only three females started egg laying not before the third day after mating. The phase of oviposition (i.e. the duration between mating and the deposition of the last egg clutch) varied broadly, ranging from 3 to 23 days (mean±SD=12.8±6.1 days). Postoviposition phase (i.e. the time between the deposition of the last cluster and the death of the female) was very long and ranged from 5 to 29 days (mean±SD=12.6±6.6 days). Longevity (excluding pre-mating phase) was 15 to 34 days (mean±SD=24.8±6.6d).

The duration of the oviposition phase was strongly correlated with fecundity and the number of egg clutches laid per female (Fig. 3).

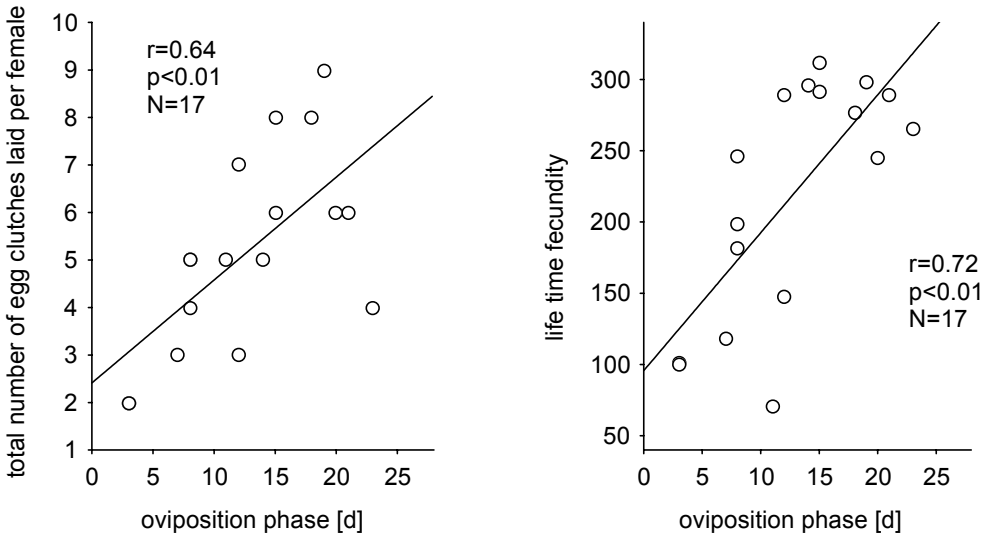

Fig. 3 – Correlation between duration of the oviposition phase (i.e. time between mating and deposition of the last egg clutch) and the number of clutches per female, or life time fecundity, respectively.

Life-time fecundity of females (total number of eggs laid) was highly significantly correlated with the number of clusters, i.e. females producing more clusters laid more eggs ($r=0.75$, $p<0.001$). However, mean clutch size per female was not influenced by its life-time fecundity ($r=0.26$, $p=0.31$). Thus, although variability in clutch size was considerable, females held the mean clutch size relatively constant except for their first clutch (mean clutch size per female, first cluster = 66.4±13.8, second and later clusters = 37.1±14.9, see Figure 4 for statistics). Egg clusters comprising less than 20 eggs were rare (8% of all clutches).

There was no correlation between the interval between two egg depositions and clutch number in the life time sequence ($r=0.06$, $p=0.60$), i.e. although there was large variability the time females needed to form a new clutch remained relatively constant (mean±SD=2.4±1.8 days). Viability of eggs decreased slightly from early to late egg clutches ($r=-0.37$, $p<0.001$) but only one female's late egg clutches were totally infertile. Total fecundity of females was not correlated with total hatching rate ($r=0.38$, $p=0.13$), i.e. females that laid more eggs did not produce proportionally fewer viable eggs.

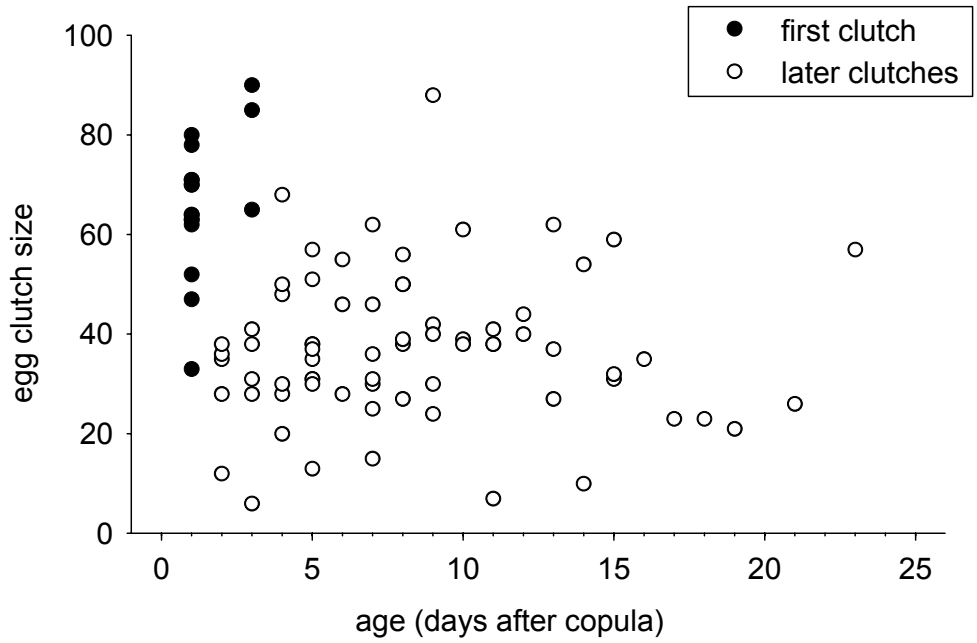

Fig. 4 – Egg clutch size as a function of female age (0=day of copula). The graph shows pooled data of all females (N=17 females, total N=87). Including first clutches, there is a negative correlation between egg clutch size and female age ($r=-0.30$, $p<0.01$). However, if first clutches are excluded age and clutch size are not correlated ($r=0.04$, $p=0.76$).

Larval survival under different conditions

Survival of caterpillars was very high under the enemy free situation in the plastic boxes (≥90%) and indistinguishable between caterpillars reared in groups of different numbers of individuals (Table 1). In contrast, survival was conspicuously lower when caterpillars were reared on potted nettles in the greenhouse. Mortality proved to be age dependent and was mainly restricted to the earlier instars, the largest fraction of larval mortality even occurring within the first days after hatching (Fig. 5, Fig. 6). Moreover, survival on potted nettles in the greenhouse strongly varied with group size. Individual larvae had lowest success (total survival: 25.6%), groups of 10 and 80 larvae experienced intermediate mortality (total survival: 63.4%, 59.0%), and groups of 40 performed best (85.3%) (Table 1).

Mortality was primarily caused by small bugs (family Anthocoridae), lacewing larvae (*Chrysopa* sp.) as well as harvestmen and spiders. Of most caterpillars which died during the experiment I found dry carcasses or parts of the body,

indicating that the rate of 'lost' caterpillars (caterpillars which could not be found since they had left the plant or which were totally devoured by predators) was low. Parasitoids were never observed during the experiments.

Table 1. Survival of caterpillars of *A. levana* reared in plastic boxes or on potted nettles in the greenhouse, respectively. Caterpillars dying during pupation were disregarded as this is an effect of overcrowding in large group sizes. Overall significance was calculated with Montecarlo statistics for R×C matrices. Significant differences for the greenhouse data were located with pairwise χ^2-tests and subsequent sequential Bonferroni correction (Hochberg 1988). Different letters in the last column indicate significant differences between groups of different size.

Group size	N groups	Total		Median per group		Probability of survival [%]	Overall p	Significant diff. between groups of different size
		Surviv.	dead	Surviv.	dead			
Enemy free situation, caterpillars in plastic boxes								
1	30	29	1	29	1	96.7	>0.05	a
10	25	224	21	9	1	90.0		a
40	15	524	59	37	3	92.0		a
80	5	361	28	76	4	95.0		a
Caterpillars on potted nettles in the greenhouse, natural enemies present								
1	82	21	61	21	61	25.6	<0.001	a
10	10	63	37	7	3	70.0		b
40	10	331	59	35	5	87.5		b
80	6	276	197	54	26	67.5		b

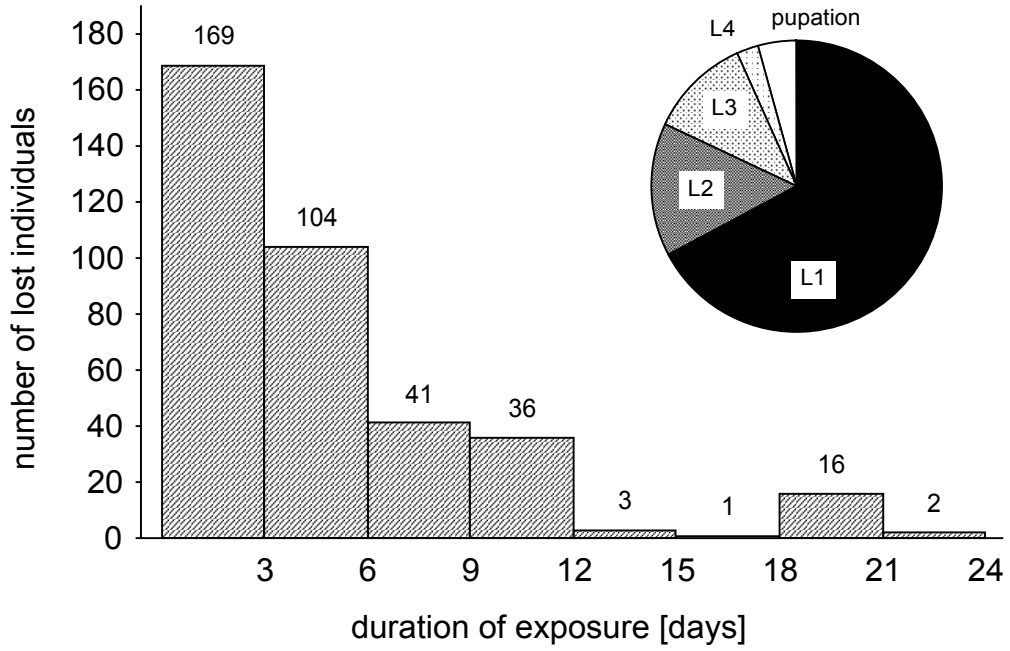

Fig. 5 – Mortality of caterpillars of *A. levana* reared on potted nettle plants in the greenhouse over the course of the experiment (pooled data for all group sizes). Inset pie-chart showing proportion of incidences of death according to developmental stage.

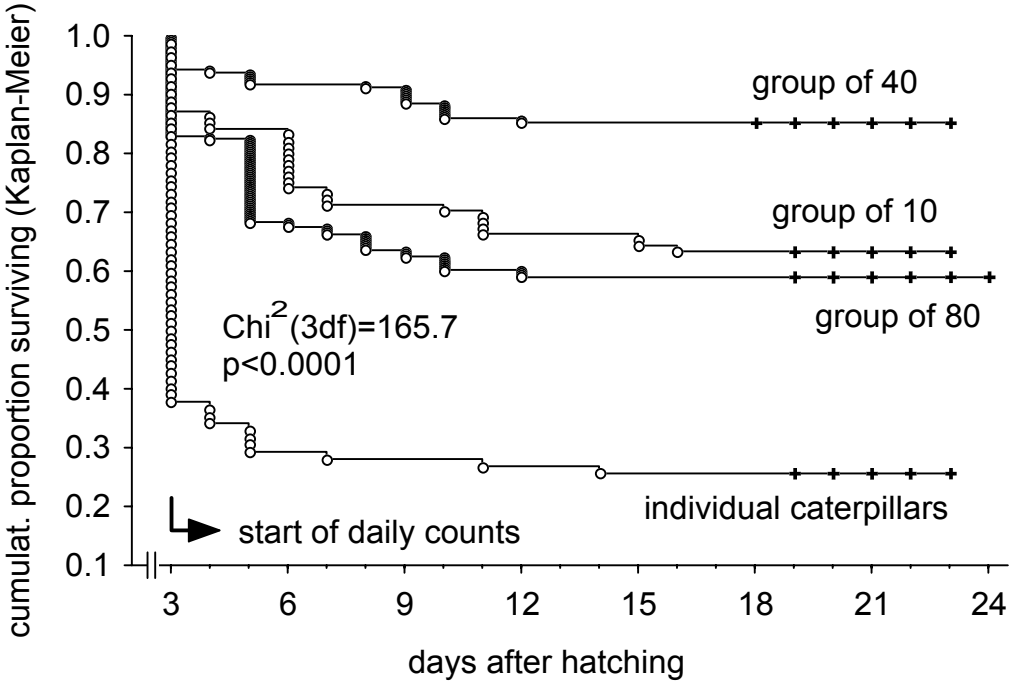

Fig. 6 – Cumulative proportion of caterpillars surviving on potted nettles (Kaplan-Meier, pooled data). Overall, survival was highly significantly differed between treatment groups (Kaplan-Meier survival analysis, $p<0.0001$). Pairwise Cox-Mantel tests show that survival was significantly higher in groups of 40 caterpillars than in groups of 10 or 80 caterpillars, and survival of single caterpillars was significantly lowest.

Survival of 30 grouped caterpillars on potted nettles exposed to field conditions was very heterogeneous (Fig. 7). Five groups went totally extinct and the maximum number of individuals surviving was only 20 (i.e. 66.7%). Although mortality rates were higher in the first instar (i.e. days 1-6, mean: 51.8%) compared to the later larval phase (mean: 22.3%, Wilcoxon matched pairs signed rank test: $Z=2.69$, $p<0.01$, groups which went extinct in L1 excluded), mortality was not largely restricted to the first instar as in the greenhouse experiment.

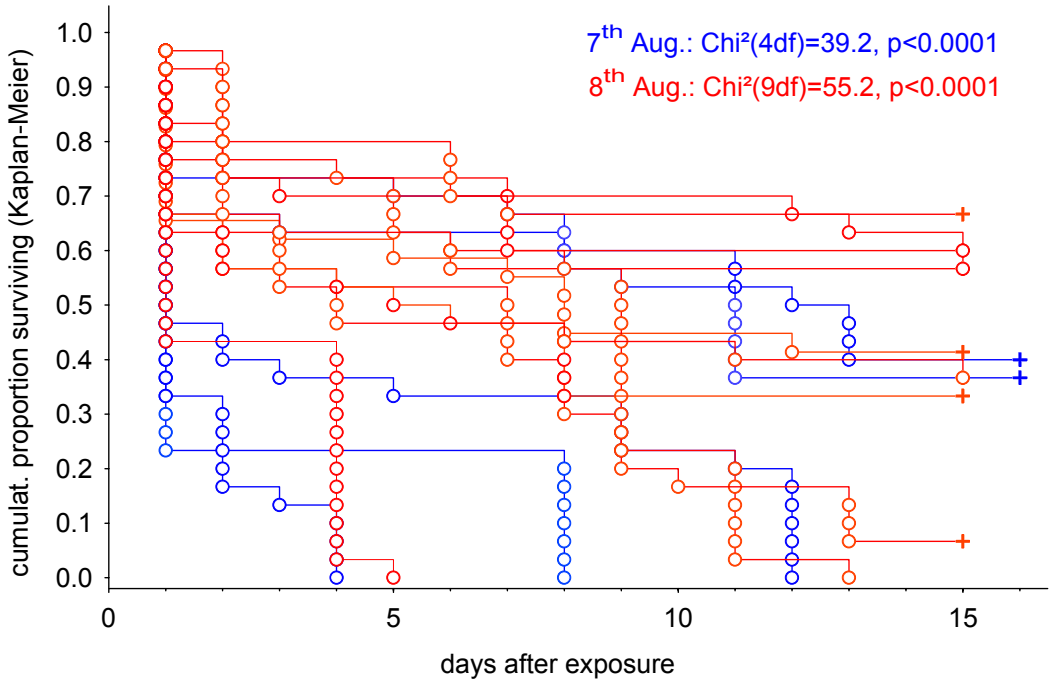

Fig. 7 – Cumulative proportion of caterpillars surviving on potted nettles under field conditions (Kaplan-Meier method; grouped caterpillars). Overall, survival highly differed significantly between the groups, irrespective of the day of exposure.

However, survival of grouped larvae was significantly higher compared to individually living caterpillars (complete extinction on four plants, maximum number of individuals surviving = 4; Fig. 8). Thus, the probability for a total extinction of the whole group was equal (Fisher's exact test: $p=0.50$), but mean survival rate was 28.9% in grouped caterpillars compared to only 4.9% in single caterpillars.

No parasitoids emerged from the larvae after they had been brought back to the laboratory.

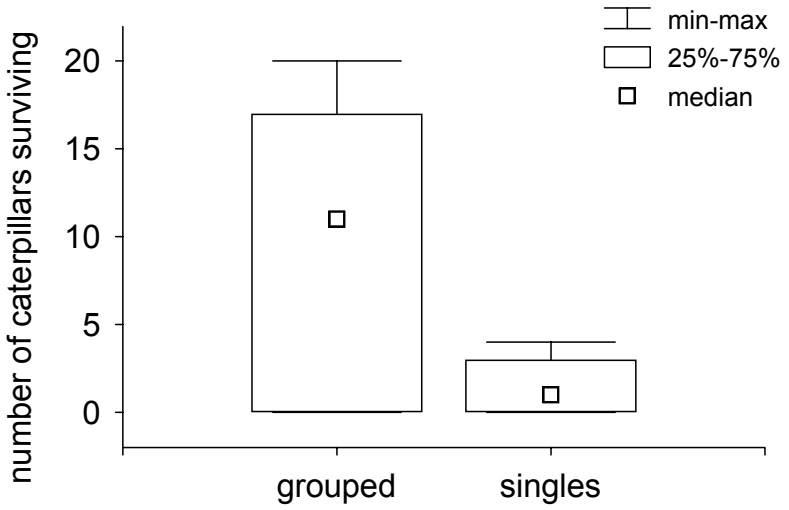

Fig. 8 – Number of caterpillars surviving on potted nettles in the field if either being grouped or spread individually over the plants. N_{start}=30 caterpillars per plant. $N_{replicates}$=15 per treatment. Survival is significantly different between groups (Wilcoxon matched pairs signed rank test: Z=2.48, p=0.01).

Defensive behavior / regurgitation

If caterpillars of *A. levana* were disturbed, they synchronously started flicking, i.e. they rose the front part of the body and moved their bodies fastly from side to side. Furthermore, a fraction of the caterpillars strongly regurgitated. At first, only small amounts of fluid formed a droplet around the mouth parts but later the whole head capsule was covered with the fluid, then appearing very large, round, and shiny black (Fig. 9, Fig. 10).

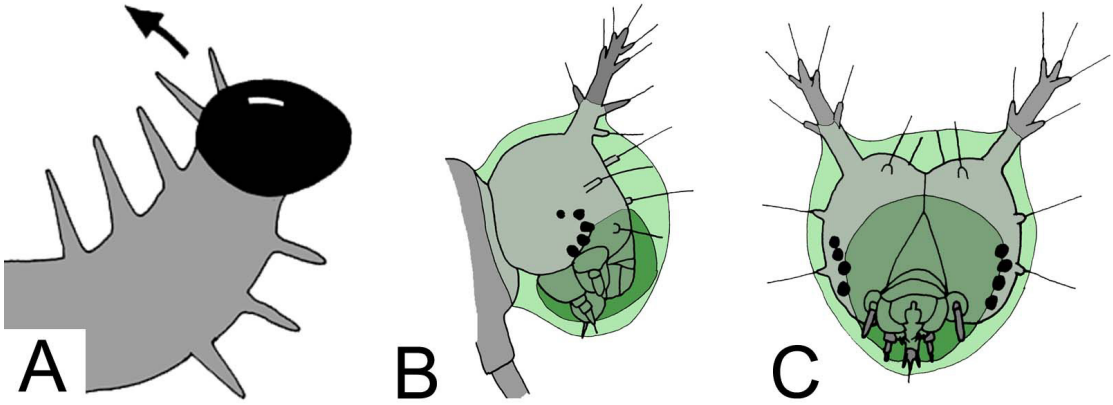

Fig. 9 – Schematized appearance of *A. levana* caterpillars during regurgitation behavior. **A** flicking behavior. Arrow indicates direction of head movements **B** lateral view **C** front view. Dark green: position of regurgitated fluid at the beginning. Light green: position of regurgitated fluid when maximally spread over the head capsule.

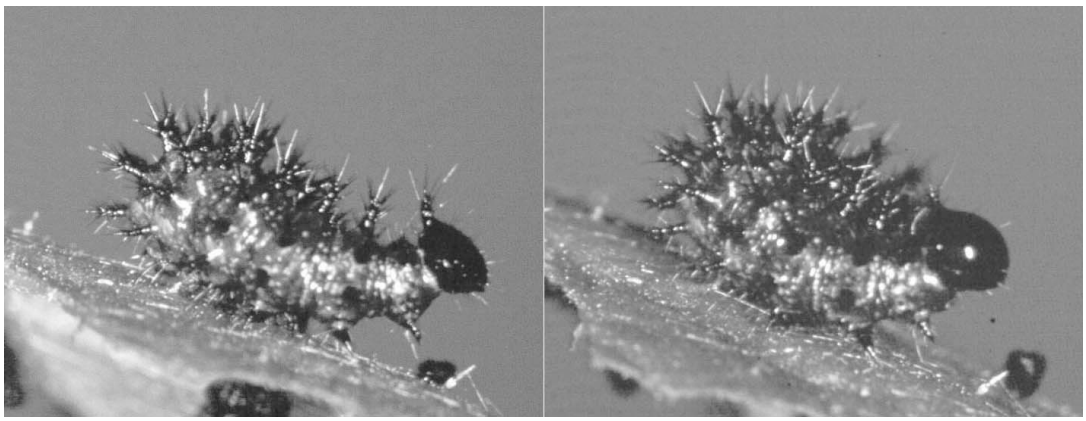

Fig. 10 – Photograph of an *A. levana* caterpillar prior to (left) and during regurgitation (right). The regurgitated fluid pours over the whole head capsule giving a shiny black, round appearance. The fluid can be re-imbibed if the caterpillar does not touch anything so that the fluid drains off the head capsule.

Caterpillars of all instars are able to regurgitate. This includes L1 larvae that are able to regurgitate immediately after hatching before the first food intake. Only caterpillars shortly before molting do not regurgitate.

However, caterpillars were not equally capable of regurgitating fluid. The volume regurgitated by different individuals was up to 13.7 times different (Fig. 11). None of the caterpillars tested did not regurgitate at all.

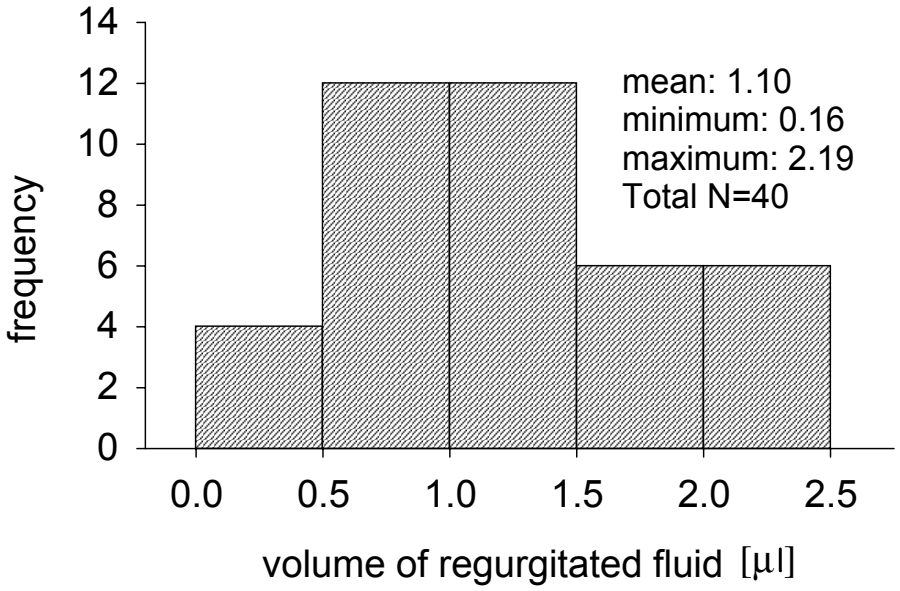

Fig. 11 – Volume of the accumulated fluid droplets regurgitated by *A. levana* caterpillars (4th instar) after simulated attacks.

The reaction of *Lasius flavus* worker ants touching the droplet with the regurgitated fluid was homogenous (Table 2). The vast majority of individuals showed marked defensive behavior and backed off the droplet immediately after touching it (Total $\chi^2(3df)=51.79$, $p<0.0001$). Many of them immediately started grooming. Only few ants showed no reaction, mainly if they possibly passed the droplet just with the legs and did not touch it with the antennae (this was not always clearly visible). None of the 40 animals in any of the three experimental replicates imbibed the fluid.

In contrast, none of the ants touching the water droplet winced and many of them rested to drink. Although the proportion of water-drinking individuals differed significantly between the three test colonies, the reaction on the regurgitated fluid was uniform (see statistics in Table 2).

Table 2. Reaction of 40 *Lasius flavus* ants encountering droplets of either regurgitated fluid or water, respectively. Statistics: Montecarlo statistics on R×C matrices to test for homogeneity across colonies (cf. Bluethgen et al. 2000).

Ant colony	Regurgitated fluid		Control (water)	
	Defense reaction	No reaction	Drinking	No reaction
1	34	6	23	17
2	38	2	31	9
3	38	2	13	27
p	p>0.05		p<0.0001	

Only 7 out of 20 *Nabis brevis* bugs were able to capture a caterpillar successfully. The remaining 13 were deterred by the flicking behavior and by the regurgitated fluid which sometimes agglutinated the antennae of the bugs. Bugs usually abandoned the attacks after two or three unsuccessful attempts and left the leaf.

Discussion

Influence of group size on survival in gregarious caterpillars

Comparing egg clutch sizes of the females from this study (first laboratory bred generation from females of the summer generation caught in 2001) to those of Chapter 12 (females from 1999), it is obvious that mean clutch size in *A. levana* is very constant (i.e. about 40 eggs. But see also discussion on geographical differences, p. 250).

This study shows that mortality of caterpillars is independent of group size when caterpillars are reared in an enemy-free situation. In contrast, solitary caterpillars and small groups are clearly at a disadvantage compared to larger groups when caterpillars were reared on intact plants with their natural enemies being present. Colonies with a group size of around 40 eggs (and later larvae) which parallels mean natural clutch size had the highest survival, suggesting that selective pressures in natural populations have favored a medium group size. This means that under an optimality framework clutch size can be considered as an adaptive trait that has been shaped to an optimal trait size by

natural selection. However, first clutches of females are usually larger than later clutches indicating that there are strong constraints affecting the female (e.g. survival probability). Thus, there are obvious conflicts of interests between the female and its offspring. While from the female's perspective the first clutch is the most valuable one (greatest likelihood of surviving up to the first egg laying, highest egg viability), the resulting very large caterpillar groups appear to be at a disadvantage relative to medium sized groups.

The influence of group size on survival of caterpillars is well documented for a number of species in the laboratory or under field conditions. However, there is no evidence for a general pattern indicating that gregariousness is always bound to advantages in survival. Results can be broadly classified in two categories:

1. Individually reared larvae or small groups of caterpillars have low survival even in enemy-free situations.

For example, Tsubaki (1981) found higher mortality of solitary individuals and smaller groups in a gregarious zygaenid moth. 20% of isolated larvae died of unsuccessful feeding in the first instar which was attributed to 'establishment mortality' (definition by Ghent 1960), i.e. solitary caterpillars are not successful to commence feeding due to their inability to produce an initial wound in the plant tissue. Similar results were found for a large number of lepidoperan and sawfly larvae (see references in Tsubaki 1981, Matsumoto 1989). Obviously, mortality during the first instar due to unsuccessful feeding in solitarily bred caterpillars and feeding facilitation in gregariously bred caterpillars is not operant in *A. levana* (see also: Suzuki & Uematsu 1981). Thus, feeding facilitation is at most of minor importance for the survival of *A. levana*, but may nevertheless be important for the caterpillars' growth (Chapter 12).

2. Individually reared larvae or small groups of caterpillars have low survival in the presence of predators and / or parasitoids.

Higher survival of larger groups under enemy pressure was found for several lepidopteran species (e.g. Morris 1976, Shiga 1976, Clark & Faeth 1997). Furthermore, comparing artificial group sizes with natural groups sizes often

revealed that survival was highest for those group sizes that resembled the naturally occurring clutch size (Stamp 1981, Matsumoto 1990). The mechanisms responsible for higher survival of grouped caterpillars are variable. Sometimes, a minimum number of caterpillars is necessary to build a leaf shelter which enhances survival (Damman 1987). Other studies found that large groups had significantly fewer predators per caterpillar than did smaller groups (so-called dilution effect; Lawrence 1990). My studies using map butterfly larvae on potted nettles in the field showed that this dilution scenario is unlikely for *A. levana*, since survival of tightly grouped caterpillars was still significantly higher than in solitary caterpillars although the total number of caterpillars per plant was kept constant. Only few studies did not find any influence of group size on survival (Denno & Benrey 1997, Fordyce & Agrawal 2001). However, optimal group size may differ for each stage of the life cycle, a consequence of changing ecological factors affecting mortality (Stamp 1981). Thus, studies focusing on life-stages that are least affected by predation or parasitism could result in misleading conclusions.

Most studies which aim at explaining the higher survival rates of groups of larvae concentrate on the communal repellent defense of grouped caterpillars as a mechanism (reviewed by Hunter 2000). The remarkably strong regurgitation behavior of *A. levana* makes it very likely that in this species, too, defensive mechanisms are responsible for the observed increase of survival rates in differently sized groups.

The efficiency of defensive regurgitation is strictly bound to the toxicity or the repellency of the host-plant material (Peterson et al. 1987, Sword 2001). Regurgitation has been studied most intensively in grasshoppers. Regurgitates of grasshoppers are a complex mixture of partially digested food, salivary secretions, and digestive enzymes (Sword 2001 and references therein). Since the regurgitated fluid of *A. levana* was not analyzed for its compounds it remains unknown what compounds cause its deterrent impact on ants or bugs. However, stinging nettles are chemically well analyzed and were shown to contain several acids (butyric acid, caffeic acid, ferulic acid) and tannins (Duke 1992) that could be responsible for the defensive effect of foregut regurgitates.

Evidence for the deterrency of regurgitate to invertebrate predators (and also birds) was provided by several studies. For example regurgitation enables grasshoppers to ward off attacking ants (Eisner 1970). Even body parts of potential prey treated with regurgitate were rejected. Wasps were reluctant to sting grasshoppers which had spread regurgitate over their bodies and showed marked grooming behavior after contact (Steiner 1981).

Defensive regurgitation has also been reported for lepidopteran and symphytan larvae. Gregarious checkerspot caterpillars (*Euphydryas* spp.) are reported to defend themselves successfully by thrashing the front half of the body and regurgitating on attacking parasitoids (Stamp 1984). Caterpillars of the lasiocampid moth *Malacosoma americanum* use allelochemicals derived from host-plant cyanogenesis for defensive regurgitation against ants (Peterson et al. 1987). Caterpillars of two solitary oecophorid species (*Myrascia* sp.*)* even possess diverticuli in the foregut to store host-plant oil for regurgitation (Common & Bellas 1977). Such diverticuli are also known from gregarious sawfly larvae (*Neodiprion sertifer*) that store host-plant resins which are used for defensive regurgitation against vertebrate and invertebrate predators (Eisner et al. 1974). Thus, all these species use compounds produced by the plant for its own defense against herbivores as a secondary defense against predators.

Defensive behaviors of caterpillars may be temporally variable. In buckmoth caterpillars (*Hemileuca lucina*) behaviors in response to simulated attacks changed markedly between the first three instars (the gregarious phase of the caterpillars) and the last three instars (the increasingly solitary phase) (Cornell et al. 1987). In the late instars caterpillars escaped from attacks by dropping or curling while the early instars caterpillars defended themselves by regurgitating and thrashing. This behavior of young larvae reflects the necessity of young larvae to react to attacks in a way as to remain in cohesion with the siblings.

The benefits of defensive behaviors may also vary depending on the predator species. Clark & Faeth (1997) found that caterpillars of the nymphalid butterfly *Chlosyne lacinia* living in large groups were well protected from solitary predators but not from ants that recruit to food. It is noticeable in this context

that defensive mechanisms are not ubiquitous in gregarious caterpillars. For example, Young & Moffet (1979) found no apparent communal defense against predators in gregarious butterfly larvae of *Mechanitis isthmia* (Nymphalidae).

The benefits of gregariousness for defensive behaviors are obvious, since many synchronously acting caterpillars may regurgitate more fluid than a single larva. Since mortality was highest during the first days after hatching, synchronized regurgitation may be particularly necessary for young larvae to provide enough regurgitate to ward off natural enemies. It is important to note in this context that the ability to regurgitate was quite variable among larvae of *A. levana*. Since no nymphalid species has ever been reported to possess foregut diverticuli to store fluid for regurgitation the ability to regurgitate will largely depend on the digestive status of each larva. Those larvae that have nearly finished digestion will not be able to regurgitate fluid from their foregut until the next feeding bout. Similar observations were made in *Hemileuca lucina* were one third of the caterpillars attacked with forceps did not show any reaction (Cornell et al. 1987). Thus, gregariousness insures the members of a group to be protected by their siblings at times when they are not ready to defend themselves.

However, it remains unclear, why very large groups of *A. levana* suffered higher mortality than medium groups. It is conceivable that large groups consume leaves very fast and are thus forced to change between foraging sites more frequently than smaller groups. These migrations will increase the caterpillar's conspicuousness and may result in temporary disintegration of the groups.

Adjustment of clutch size and female fecundity

If survival is highest in groups of a medium size (about forty caterpillars in *A. levana*), why is there so much natural variation in batch sizes? Since the pioneering work of Lack (1947, 1948) a great deal of interest has centered on the evolution of clutch size in birds. However, clutch size in birds is dominated by the influence of parental care. Models for clutch size determination in animals without parental care have been developed in the 1980's (e.g. Parker &

Courtney 1984, Skinner 1985, Mangel 1987). Some of the most important aspects and predictions from the models are addressed below.

1. Selective pressures on the caterpillars may vary temporally and spatially (cf. discussion p. 245). For example, caterpillars in other geographical regions may face other selective pressures with another optimum clutch size. Danish *A. levana* reportedly lay clutches of over 100 eggs (Henriksen & Kreutzer 1982) which is above the maximum clutch size of *A. levana* as observed in my experiments with animals from the vicinity of Bayreuth. In contrast, Japanese Map butterflies reportedly lay only 4 to 20 eggs (Fukuda et al. 1991). Natural groups of Belgian *A. levana* are reported to contain 10-30 individuals (Maes & Van Dyck 1999) which is comparable with our data if mortality in the early instars is considered. Thus, variability may be a strategy to spread the risk due to a relative unpredictability of the pressure imposed by predation and parasitism.

2. Optimal clutch size is influenced by possible competition among the hatchlings for resources (Parker & Begon 1986). Therefore, females could lay larger clusters on hosts that can support the growth of more offspring (Pilson & Rausher 1988, Damman 1991, Tsubaki 1995). In *A. levana*, however, competition for food is highly unlikely because stinging nettles build rhizomes and individual clones thus usually cover several square-meters at suitable sites (nutrient rich soil, high humidity – these are the typical larval habitats of *A. levana*: ProNatura 1987, Ebert & Rennwald 1993). Thus, competition among siblings has never been reported for the map butterfly and will consequently hardly influence clutch and group size.

3. Clutch size is not only influenced by maximization of surviving larvae but also by the life-expectancy, resource availability and potential fecundity of the female (Parker & Courtney 1984, Mangel 1987, Minkenberg et al. 1992). For example, if the expectation of surviving to lay a second clutch is low, more eggs should be laid in the first clutch. The validity of this prediction has been shown for other nymphalid butterflies as well (e.g. Warren et al. 1994, Wahlberg 1995) and is also consistent with my data. Furthermore, clutch size is predicted to decrease

as the number of eggs remaining to be laid becomes low (Forsman 2001). However, in *A. levana* clutch size and time between two clutches remained relatively constant over time (excluding the first clutch). Map butterflies hatch with a mean number of ca. 60 mature eggs (Bink 1992). As in many other butterflies new eggs mature during the oviposition period (cf. Wheeler 1996), i.e. map butterflies are income breeders (Tammaru & Haukioja 1996). Thus, life-time fecundity is strongly correlated with longevity. Consequently, it is very likely that females invest most of the available eggs into the first clutch and are then limited by the time required to mature further eggs.

In the laboratory females of *A. levana* survived for 2-5 weeks but the oviposition phase spanned only 3 to 23 days. Although fertility decreased with age, *A. levana* females are probably not sperm limited because females that had a high life time fecundity did not show reduced hatching rates. However, resource limitation might have played a role if the offered nectar plants and water resources did not fulfill the female's requirements (i.e. there were constraints on egg maturation and vitellogenesis. For a general discussion of the importance of adult nutrition for egg production see Boggs 1986). The long post-oviposition phase is probably influenced by artificial conditions in my experiments with nectar plants and water being available *ad libitum* in close proximity to the females that would otherwise probably have died faster. Unfortunately, there are no data on the life expectancy of Map butterflies under field conditions. Summer populations of the field generation were reported to show an open population structure and females had low site tenacity (Fric & Konvička 2000). Thus, even rough estimations of survival probabilities under field conditions are difficult to obtain. Even rough estimates of life-time fecundity are rare in the literature. Bink (1992) stated, without reporting sample sizes or details as to how he obtained his data, that maximum egg production ranges between 250-280 eggs. This is slightly higher than the average I observed (219 eggs), but lower than the maximum I recorded (298 eggs).

What are the major benefits of gregariousness in A. levana*?*

Caterpillars of *A. levana* reared in groups on potted nettle plants in the greenhouse had shorter developmental times than solitary caterpillars (Chapter 12) *and* showed reduced predation rates. Thus, it remains debutable which of these benefits is more important. Do grouped caterpillars show higher survival rates due to faster development (presumably by better overcoming of plant defense structures), or are survival rates higher due to a more effective predator defense? Of course, mechanisms of increased development time and reduced predation are not mutually exclusive. Faster growth rates may decrease mortality by reducing the time during which especially the small larvae are most vulnerable to predation (Lawrence 1990). However, my studies in the greenhouse showed that developmental benefits were rather low whereas survival rates differed markedly. Unfortunately, high temperatures resulting in overall high growth rates limited the potential for a plastic response that was expected in this experiment.

There are of course other potential benefits of gregariousness (cf. Chapter 1). Benefits by enhanced thermoregulation are most unlikely, since caterpillars of *A. levana* live on the underside of stinging nettle leaves and prefer shaded habitats for egg deposition (Ebert & Rennwald 1993). Furthermore, constraints on females like a lack of nectar plants and restricted availability of host-plants are also unlikely. Thus, I conclude that the main benefits of gregariousness in *A. levana* accrue from synchronized, communal predator defensive behaviors. Theoretical work suggests that repellent defenses should be an important precondition for the evolution of gregariousness in many insects (Sillén-Tullberg & Leimar 1988). These assumptions are confirmed by recent phylogenetical studies which show that gregariousness is more likely to evolve in lineages with repellent defense (and in lineages with warning coloration; Tullberg & Hunter 1996). However, it remains and unresolved puzzle why gregariousness is particularly common in many clades of Nymphalidae, including the Nymphalini (to which *A. levana* belongs). Further studies on a wider range of nymphalid butterfly species combined with phylogeny-based comparative approaches will be necessary to finally address this question.

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Chapter 14

Synopsis

When most biologists think of social animals, caterpillars not even play a minor role in their considerations. Some most recent reviews and books focusing on animal groups or sociality also still concentrate exclusively on vertebrates (Johnson et al. 2002, Krause & Ruxton 2002). However, many gregarious caterpillars exhibit more strongly integrated group behaviors than many vertebrates considered to be social (e.g. fish schools, Wittenberg 1981), for example communal shelter building or trail-based communication. Even among entomologists social insects (and other arthropods) apart from ants, bees, wasps and termites were at the most considered 'presocial' (Eickwort 1981) for many decades. However, recent reviews and books have given more attention to social arthropods that do not fulfill the criteria of eusocial insects (e.g. Fitzgerald & Peterson 1988, Choe & Crespi 1997, Costa 1997).

Sociality in caterpillars: benefits and mechanisms

This thesis comprises ten chapters on different aspects of sociality or gregariousness in caterpillars. With regard to the questions derived in Chapter 1 the following conclusions can be drawn for the two main focal species under consideration.

In *Eriogaster lanestris* **advantages** accruing from sociality proved to be numerous. Major benefits of sociality obviously emerge from the combined effects of the tent and collective warming. Large, dense tents, unlike thin webs, can only be built communally by large numbers of caterpillars and are very effective in reducing convective heat loss. Simultaneously, caterpillars benefit from social thermoregulation by endogenous means and by basking (Chapters 4+5). Developmental benefits through higher body temperatures obviously outweigh the high costs of sociality (Chapter 10).

In order to benefit from these advantages **ethological preconditions** are required. *E. lanestris* caterpillars proved to have a highly sophisticated

communication system which enables caterpillars to still forage effectively in an environment where available food becomes more and more patchily distributed during the course of the caterpillars' extended social phase (Chapters 7+8). Moreover, it was shown that caterpillars exhibit a high degree of synchronicity during the foraging bouts which seems especially crucial for trail marking (Chapter 6). However, while sophisticated trail-based communication emerges as a general property of central-place foraging as well as nomadically foraging social caterpillars, behavioral synchronicity does not necessarily occur in species with such foraging systems. *E. catax* and *M. neustria*, though rather closely related in phylogenetic terms to *E. lanestris*, only partially synchronized their activities (Chapter 11).

The **mechanisms** underlying these behaviors are now fairly well understood for communicative aspects (by identifying the trail marking mode and the possible encoding of information in the trails by quantitative graduation of the trail marker, Chapter 8). The hypothesis that the mechanism for behavioral synchronicity lies in communication through substrate-borne vibrations is now even more plausible since I showed concrete data supporting this idea for the first time.

Despite their black coloration caterpillars of *Araschnia levana* do not thermoregulate but main **advantages** of their social life-style are an enhanced development and a higher survival rate compared to single caterpillars. Group cohesion as a main **precondition** for gregariousness was shown to be achieved by trail following behavior. The **mechanisms** for the enhanced development by social feeding facilitation are not yet understood. Though, higher survival is achieved by group defense, with enhanced collective regurgitation being the most prominent component.

General patterns?

This study has shown for a very small set of Lasiocampid species that foraging patterns in caterpillars differ markedly. Foraging patterns of Lepidopteran caterpillars as a whole are even much more diverse (e.g. diurnal vs. nocturnal,

endophagous vs. ectophagous), and there is a remarkable diversity of color patterns (ranging from blending into the background and mimicking inedible objects to displaying bright, colorful signals), size, and thermal behavior (regulators or conformers) (Stamp & Casey 1993). Such diversity in traits affecting foraging exists despite the seemingly simple set of constraints on larvae: Caterpillars, within their limitations as ectotherms, must avoid their natural enemies while obtaining as much food for growth as possible in a minimum time. Searching for a common pattern even among closely related species is critical because besides phylogenetic factors, biotic and abiotic constraints specific to a species' environment shape the foraging pattern and are reflected in the caterpillars' life-style.

Several characteristics of lepidopteran feeding, such as host specificity, degree of gregariousness, degree of conspicuousness, and position or time of feeding are potentially influenced by selection pressures from natural enemies (Montllor & Bernays 1993). Taken together for any given species, these and other related features define a foraging strategy. Presumably each of these diverse strategies seen in nature are a compromise between conflicting pressures, a balance of the costs and benefits incurred by feeding in a particular way on particular plants.

Studies on *Malacosoma americanum* (Fitzgerald 1980, Fitzgerald et al. 1988), *E. lanestris* (Ruf et al. 2001, Ruf & Fiedler 2002), and many nocturnal social species (see overview in Ruf & Fiedler 2002) led to the expectation that synchronicity during foraging is a general trait in social caterpillars. However, this study showed that despite numerous shared life-history traits very different patterns of activity and foraging may arise (Chapter 11). The developmental consequences of feeding rhythms have rarely been addressed. Some few studies (Herrebout et al. 1963, Slansky 1974, Chapter 6) suggest that feeding rhythms, whatever the selective forces molding their evolution, likely have substantial ramifications for caterpillars' food consumption, growth, and associated life cycle components (e.g. number of generations per year and diapause stage), but clearly much more data are required to assess this more thoroughly. One conclusion clearly emerges from the data presented here and

integrated into the existing body of evidence: Even among taxonomically related and ecologically similar species with central-place foraging larvae, many generalizations expected from earlier studies (e.g. strict synchronicity, fixed foraging schedules) do not hold. Thus, only when considering the phylogenetic position plus all ecological forces simultaneously, it is possible to really understand the diversity of solutions that social Lepidopteran larvae have achieved during evolution.

Evolution of sociality in caterpillars

The evolution of egg clustering and gregariousness among caterpillars has generated a long and yet unresolved debate. Since all social caterpillars are derived from cases where eggs are laid in clusters, many investigators dealing with the evolution of gregariousness in caterpillars have focused on elucidating the egg clutch laying habit (e.g. Stamp 1980, Young 1983) and on selection pressures operant on the adults. It was suggested that batch oviposition is particularly likely to arise if ovipositional plants are rare and thus females seldom encounter larval food plants. Moreover, selection pressures from predators capturing females, inclement weather that limits egg laying, and scarcity of adult nectar plants may favor cluster oviposition (Stamp 1980). Furthermore, clustering may render eggs less vulnerable to desiccation (Clark & Faeth 1998).

It is a controversial issue, whether the apparent benefits accruing to aggregated larvae are consequences rather than causes of the evolution of egg clustering. For example, batch laying may increase realized fecundity (Courtney 1984). Thus, increased fecundity could be the main factor underlying the evolution of cluster oviposition. Consequently, cluster oviposition would have arisen first and would have set the stage for the evolution of larval adaptations that could eventually lead to sociality.

However, if larval success depends on group size it seems reasonable to suppose that fitness enhancing aspects of gregarious behavior should contribute to the evolution of adult ovipositional patterns. Several chapters of

this thesis have indeed shown that benefits accruing from gregariousness depend on having a large number of caterpillars (in *E. lanestris*: e.g. Chapters 4,5,9) or on the particular size of the groups (in *A. levana*, Chapters 12+13). However, only extensive phylogenetic studies will elucidate the sequence of events in evolutionary pathways leading to caterpillar sociality (e.g. Tullberg & Hunter 1996) and it is very likely that there are different pathways in different families.

Fitzgerald & Costa (1986) hypothesized for social Lasiocampidae (*Malacosoma* spp.) that the trail laying behavior of solitary ancestral species led to encounters of individuals. Benefits accruing to these incipient aggregates could then have favored females that laid their eggs in groups. However, in the Lasiocampidae that have no functional proboscis and are obviously selected for maximizing fecundity (i.e. capital breeders sensu Tammaru & Haukioja 1996), females are very short-lived, have a high wing load, and consequently under manifold ecological conditions benefit from laying all eggs in one cluster irrespective of the advantage accruing to their offspring (see also Ruf & Fiedler 2000 for further discussion). However, in the social species caterpillars hatching from these eggs do not disperse like it is common in many solitary Lasiocampidae (e.g. in the genera *Gastropacha*, *Macrothylacia*, Ebert 1994) or Lymantriidae (e.g. in the genera *Lymantria*, *Orgyia*, Ebert 1994). Hence, whatever the evolutionary mechanism is, it seems reasonable to suppose that the benefits accruing to the caterpillars should at least stabilize the female's egg deposition habits.

In *A. levana* there are no such obvious morphological constraints that could explain the egg batch laying habit as in the Lasiocampidae. The proboscis of this species is normally developed, adults are long-lived (Chapter 13) and they do not hatch with all eggs being matured at eclosion. Moreover, many more species in the tribes Nymphalini and Melitaeini lay their eggs in clusters (Ebert & Rennwald 1993) which are also very long-lived and have a normally developed proboscis (e.g. such common species as *Inachis io*, *Aglais urticae*). Since females of egg clustering nymphalid species always lay several clusters, they spread the risk of losing all their offspring when one clutch completely fails. Nevertheless, grouped caterpillars have much higher survival rates which

makes it most likely that the caterpillars' success will again influence the egg laying habits of the females.

In *E. lanestris* large colonies develop faster since more individuals can build larger tents and have better capacities for social thermoregulation (cf. Chapters 4+5). If one disregards potential problems of resource overexploitation (which are effectively counterbalanced by trail communication and recruitment to profitable food finds) the strategy here must be 'more is better' (i.e. the maximally achievable group size is the best). In contrast, *A. levana* caterpillars rely on a hidden life-style. Provided that the main benefits of gregariousness in this species come from collectively warding off natural enemies it seems logical that there is an intermediate group size which optimizes defensive behaviors whereas larger group sizes increase the risk of being detected. Thus, optimal group size corresponds to the maximum of a bell-shaped clutch-size curve.

To the extent that the field studies conducted in the course of this thesis are representative (Chapter 10+13) costs of sociality in *E. lanestris* are very high since half of the females lost their whole offspring. On the other hand, colonies with mature caterpillars found in the field regularly contained about 250 caterpillars (i.e. about 80% of the egg number of an average cluster). This indicates that benefits (e.g. by social thermoregulation and potentially by group defense) may be also very high and thus outweigh the costs on the population level. In *A. levana* maximum survival in the field was only 67% of the individuals of a group and 33% of the groups also totally failed, but multiple batch laying decreases a female's risk to lose her whole offspring. Possibly, the lower maternal risk also leads to smaller benefits.

Being gregarious may reduce some constraints on foraging but imposes others. Thus, research on behavioral strategies of caterpillars must take more of a multiple factor approach. The studies included in this thesis have contributed to complete some gaps in our current knowledge on sociality in larval Lepidoptera. Moreover, it was shown that studies testing widely hold hypotheses with hitherto under-studied species is still promising and may bring out new, and partly unexpected patterns. Many more studies on different species with gregarious

caterpillars combined with phylogenetic analyses will be necessary to fully understand how behavioral mechanisms and ecological consequences interact with the evolution of sociality in Lepidopteran caterpillars.

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Summary

Preliminary note: Please notice that Chapters 4–13 are accompanied by their own abstracts.

The present thesis investigates ecological advantages, ethological adaptations, and behavioral mechanisms connected with sociality in larval Lepidoptera. Data on social thermoregulation, larval communication, developmental benefits, and advantages of survival are presented to contribute to a better understanding of the evolution and maintenance of social systems in caterpillars.

I put the main emphasis of this work on the highly social, tent building European lasiocampid moth *Eriogaster lanestris*. In a comparative study aspects of the social biology of *E. lanestris* were compared to its congener *E. catax* and the confamilial species *Malacosoma neustria*. In addition to these tent building, central-place foraging species a fourth, non-tent building nomadic butterfly species, *Araschnia levana* (Nymphalidae) was also studied.

- Caterpillars of *E. lanestris* are behavioral thermoregulators. In the laboratory (i.e. in the absence of solar radiation) tightly aggregated caterpillars are able to rise their body temperatures compared to ambient temperature for 2.5-3K. Temperature gains are based on metabolic heat production (Chapter 4). Under field conditions grouped caterpillars are able to stabilize their body temperatures between 30-35°C independent of ambient temperature as long as solar radiation is sufficient. Optimal body temperatures are achieved by changing positions in and on the tent frequently. The functionality of the tent is based on the reduction of convective heat exchange (Chapter 5).
- Foraging bouts of *E. lanestris* caterpillars are highly synchronized under field conditions. The number of foraging bouts is temperature dependent (influence on speed of digestion and walking). Plasticity in foraging patterns optimizes nutrient uptake and therefore minimize developmental time (Chapter 6).
- Caterpillars of *E. lanestris* use trail pheromones for communication. The pheromone is applied by dragging the ventral median sternite of the last abdominal segment over the substrate. Trails contain information about age

and suitability of foraging sites, which is encoded by quantitative graduation of the trail marker (opposing processes of aging and (over)marking) (Chapters 7+8).

- Vibrations of the tent caused by the increasing restlessness of caterpillars at the end of the digestion phase turned out to be tightly linked to the emergence of larvae from the tent and are supposed to play a role for synchronizing the foraging bouts (Chapter 9).
- Costs of social behavior in *E. lanestris* are high under field conditions. Total colony mortality (i.e. the complete loss of a female's offspring) of 100 exposed colonies was 48%. The majority of colony losses was connected to the previous loss of the tent, mostly initiated by heavy rainfalls (Chapter 10).
- Comparing *E. lanestris* to *E. catax* and *M. neustria* revealed that despite similar life-history traits and overlapping habitat requirements very different ecological strategies evolved with regard to egg size, tent building behavior, foraging strategies, and activity patterns (Chapter 11).
- Females of *A. levana* produce several egg clutches of different size over their life time. Caterpillar group size strongly influences development and survival. Single individuals develop more slowly and achieve smaller weights compared to groups of 10, 40 (=mean natural clutch size), or 80 individuals during the social phase of the caterpillars' life (i.e. first to third instar). Mortality is highest in singly bred individuals and lowest in groups of 40. The advantage of grouping for survival is supposed to result from the strong collective regurgitation behavior which enables the caterpillars to ward off natural enemies (Chapters 12+13).

The thesis shows with the example of *E. lanestris* that highly evolved social systems in larval Lepidoptera afford high parental and larval investment which may in turn lead to high advantages, e.g. with regard to large reductions in developmental time. The extended social phase requires highly sophisticated communication processes. In *A. levana* flexible egg deposition leads to higher plasticity with regard to the costs of sociality. Nevertheless, physiological and ecological advantages of group living are evident as well.

Zusammenfassung

Neben den traditionell als „sozial“ oder „eusozial“ betrachteten Insekten (Ameisen, Bienen, Termiten) findet sich in der moderneren Literatur ein zunehmendes Interesse an solchen Insekten und anderen Arthropoden, die nicht durch die klassischen Kriterien wie reproduktive Arbeitsteilung, kooperative Brutpflege und überlappende Generationen gekennzeichnet sind, dennoch aber klare Grundzüge sozialen Verhaltens (z. B. enger Gruppenzusammenhalt, Kommunikation der Gruppenmitglieder untereinander) zeigen. Solche Sozialsysteme sind i. d. R. nicht durch Verwandtenselektion geprägt, sondern stellen in erster Linie durch ökologische Zwänge bestimmte Zweckgemeinschaften dar. Es ist das Ziel dieser Dissertation, das noch auf wenige Arten beschränkte Wissen über in diesem Sinne sozial lebende Raupen zu erweitern und wichtige Hypothesen zur Funktion und Ökologie solcher Gemeinschaften zu testen.

Der Schwerpunkt der Arbeit wurde dabei auf das Sozialverhalten der Raupen des paläarktisch verbreiteten Wollafters *Eriogaster lanestris* (Lasiocampidae) gelegt, da diese Art ein außergewöhnlich hohes Maß an Komplexität bezüglich ihres Sozialverhaltens zeigt und die Larvengemeinschaften bis fast unmittelbar vor dem Abschluß der Larvalentwicklung zusammenbleiben, wobei sie ein gemeinsam erbautes Seidenzelt bewohnen. In einer vergleichenden Studie wurden Teilaspekte des Sozialverhaltens von *E. lanestris* mit zwei weiteren, zeltbauenden Lasiocampiden-Arten, dem Hecken-Wollafter *Eriogaster catax* und dem Ringelspinner *Malacosoma neustria* verglichen. Im Gegensatz zu diesen drei zeltbauenden Arten ist der Sozialverband der Raupen der vierten untersuchten Art, des Landkärtchens *Araschnia levana*, schwächer: Die Raupen leben nur in den ersten drei Larvenstadien gregär und investieren keine Ressourcen in den Bau eines Seidenzelts. Es war deshalb zu erwarten, daß sich bei *E. lanestris* zahlreiche meßbare Effekte der Sozialität auf verschiedene Aspekte des Lebenszyklus zeigen, während bei *A. levana* die Vorteile des Sozialverbandes weniger stark ausgeprägt sein sollten.

Die vorliegende Arbeit verfolgte dabei drei Schwerpunkte:

(1) Messung und Beschreibung der ökologischen Vorteile sozialen Verhaltens

(2) Analyse der ethologischen Voraussetzungen, die es überhaupt möglich machen, von diesen Vorteilen zu profitieren

(3) Analyse der Mechanismen der für die Sozialität relevanten larvalen Verhaltensmuster

Die folgenden Ergebnisse werden in den einzelnen Kapiteln vorgestellt:

- Durch hochsynchronisierte Fouragierperioden kommt es bei *E. lanestris* stets auch zur Klumpung der Raupen im oder auf dem Zelt während der Ruhe- / Verdauungsphasen. Dabei erzielen die Raupen unter Laborbedingungen um 2,5–3 K erhöhte Körpertemperaturen im Vergleich zur Umgebungstemperatur. Da im Labor keine Energieaufnahme durch Absorption von solarer Strahlungsenergie erfolgen kann, muß diese Erwärmung auf metabolischer Wärmeproduktion beruhen (Kapitel 4).

- Unter Freilandbedingungen können *E.-lanestris*-Raupen ihre Körpertemperatur weitgehend unabhängig von der Außentemperatur zwischen ca. 30 und 35 °C stabilisieren, solange die Sonneneinstrahlung ausreichend hoch ist. Dies wird vor allen Dingen durch gezielte Positionswechsel im und auf dem Seidenzelt erreicht. Die Seide schirmt die einfallende Strahlung weitgehend ab. Die Funktionalität des Zeltes beruht also offensichtlich vor allen Dingen auf der Verminderung konvektiven Wärmeaustauschs und nicht auf einem Treibhauseffekt, wie dies häufig propagiert wurde (Kapitel 5).

- Durch elektronische Langzeitüberwachung von *E.-lanestris*-Kolonien konnte gezeigt werden, daß auch unter Freilandbedingungen hochsynchronisierte Fouragierperioden stattfinden. Dabei ist die Anzahl der täglich stattfindenden Fouragierperioden stark temperaturabhängig, da hohe Temperaturen sowohl die Verdauungszeit (Intervall zwischen den Fouragierperioden) als auch die Fouragierperioden selbst (durch beschleunigte Lokomotion) verkürzen. Solche plastischen Fouragiermuster optimieren die Nahrungsauf-

nahme und folglich die Entwicklungsdauer und konnten erstmals für eine soziale Schmetterlingsart gezeigt werden (Kapitel 6).

- Die Kommunikation der Raupen von *E. lanestris* erfolgt mittels eines Spurpheromons, das von der Ventralseite zwischen den Nachschieberbeinen abgestreift werden kann. Die Raupen bevorzugen neue gegenüber alten Spuren. Ferner bevorzugen sie solche Spuren, die von Raupen gelegt wurden, die erfolgreich eine Futterquelle erschlossen haben, gegenüber solchen Wegen, die von Raupen gelegt wurden, die erfolglos und hungrig zum Zelt zurückkehrten. *E. lanestris* zeigt Spurfolgeverhalten auf Wegen, die mit 5β-Cholestan-3-on präpariert wurden, einer Substanz, die für die verwandte Art *Malacosoma americanum* als Teil des natürlichen Spurpheromons nachgewiesen wurde. Höher konzentrierte Spuren werden gegenüber schwächeren bevorzugt; die verhaltensauslösende Schwellenkonzentration liegt bei 10^{-10} g/cm (Kapitel 7).

- Raster- und transmissionselektronenmikroskopische Aufnahmen des Bereichs zwischen den Nachschieberbeinen von *E. lanestris* zeigen keine auffälligen Drüsenzellen. Offensichtlich wird das Spurpheromon von in der Epidermis verteilten, einzelnen spezialisierten Zellen produziert. Die Auftragung erfolgt während jedes Bewegungszyklus durch Abstreifen der ventralen Integumentfläche zwischen den Nachschiebern. Die Raupe kann durch schnelles Hochziehen des Nachschiebers die Markierung während des Laufvorgangs aussetzen. Die Attraktivität einer Spur wird vermutlich nur durch quantitative Abstufungen der Spurmarkierung erzielt, da Raupen nicht fähig sind, künstlich abgestreiftes Spurpheromon von satten bzw. hungrigen Raupen zu unterscheiden. Der Wechsel von einer ehemals attraktiven Spur auf eine neue vollzieht sich während des Fouragierprozesses einer Kolonie nur langsam durch eine Alterung und ausbleibende Neumarkierung des entsprechenden Wegs (Kapitel 8).

- Für die Synchronisierung der Kolonieaktivität während der Fouragierperioden können die Vibrationen des Zelts eine Rolle spielen, die durch die Unruhe der Raupen am Ende einer Ruhe- und Verdauungsphase zustande

kommen. Das Auslaufen der Raupen erfolgt exakt zu dem Zeitpunkt, an dem die Zunahme der Zeltvibrationen maximal ist, d. h. wenn die Mobilisierungsrate der Raupen im Zelt am höchsten ist. Kleine Kolonien sind vergleichsweise schlecht synchronisiert und zeigen eine um mehrere Tage verzögerte Entwicklung bei ansonsten identischen Umweltbedingungen (Kapitel 9).

- Die Kosten sozialen Verhaltens bei *E. lanestris* unter Freilandbedingungen sind hoch. In einem Feldexperiment starben 48 von 100 exponierten Kolonien aus, was jeweils den Komplettverlust der Nachkommenschaft eines Weibchens bedeutet. Von diesen Verlusten waren 71 % mit dem Verlust des Zeltes gekoppelt, wobei sich Starkregenereignisse als besonders zerstörerisch für die Seidenzelte erwiesen. Dies unterstreicht die besondere Bedeutung des Zelts für das Überleben und die Entwicklung der Raupen, aber auch die Risiken, die die weitgehende Abhängigkeit von der Intaktheit des Zeltes mit sich bringt. Raupen *E. lanestris* ergänzen beschädigte Seidenzelte, bauen aber i. d. R. kaum je ein Ersatzzelt. Die Mortalität der Kolonien war über die gesamte Larvenentwicklungszeit weitgehende gleichmäßig verteilt (Kapitel 10).

- Ein Vergleich von *Eriogaster lanestris* mit *E. catax* und *Malacosoma neustria* hinsichtlich verschiedener Life-history-Eigenschaften und ihres Verhaltens zeigt, daß Gemeinsamkeiten und Unterschiede nicht an die Verwandtschaftsverhältnisse der Arten gekoppelt sind. Trotz stark überlappender Habitatansprüche entwickelten alle drei Arten sehr verschiedene Strategien, z. B. hinsichtlich Eigrößen, Zeltbau, Fouragierstrategien und Aktivitätsmustern (Kapitel 11).

- Bei *Araschnia levana* hat die Gruppengröße einen massiven Einfluß auf die Entwicklung der Raupen: Einzeln gehaltene Tiere zeigen während der ersten drei Larvenstadien (entspricht der gregären Phase) eine geringere Wachstumsrate, sind als L3-Larven leichter und entwickeln sich langsamer als Tiere in Gruppen (je 10, 40, 80 Individuen), wenn sie unter prädatorenfreien Bedingungen mit Futter *ad libitum* aufgezogen werden. Die Effekte bei

der Aufzucht auf getopften Futterpflanzen und in Anwesenheit natürlicher Prädatoren waren tendenziell gleich, aber nicht immer signifikant (Kapitel 12).

- Weibchen von *A. levana* produzieren mehrere Eigelege, wobei das Erstgelege signifikant größer ist als die Folgegelege. Auf getopften Futterpflanzen im Gewächshaus gehaltene Raupen zeigen eine hohe Mortalität während der ersten drei Larvenstadien, wobei die Überlebenswahrscheinlichkeit bei einer Gruppengröße von 40 Individuen (entspricht der mittleren natürlichen Eigröße) am höchsten ist, während Tiere in Gruppen à 10 oder 80 Individuen eine signifikant höhere Mortalität zeigen und Einzeltiere die höchste Sterblichkeit erfahren. Der Gruppenzusammenhalt wird durch ein (nicht näher identifiziertes) Spurpheromon vermittelt. Die erhöhte Überlebenswahrscheinlichkeit kann auf das starke gemeinschaftliche Feind-Abwehrverhalten der Raupen (Regurgitieren) zurückgeführt werden (Kapitel 13).

Die Studie zeigt am Beispiel von *E. lanestris*, daß hochkomplexe Sozialverbände von Raupen zum einen hohe Investitionen fordern (hohe parentale und larvale Investition, komplette Abhängigkeit der Individuen vom Sozialverband), allerdings auch zu großen Vorteilen, z. B. hinsichtlich einer starken Verminderung der Entwicklungszeit, führen. Dabei erfordert das lange Zusammenleben auch komplexe Verhaltensmuster und Kommunikationsprozesse.

Bei *Araschnia levana* zeigt sich, daß eine flexible Eiablage-Strategie höhere Plastizität hinsichtlich der Kosten der Sozialität bieten und Sozialität auch nur auf frühe Larvenstadien beschränkt sein kann. Dennoch sind auch in diesem Fall physiologische und ökologische Vorteile des Gruppenlebens evident.

List of publications

Publications in peer reviewed journals

RUF, C.; FIEDLER, K. (2000) Thermal gains through collective metabolic heat production in social caterpillars of *Eriogaster lanestris*. *Naturwissenschaften* 87, 193-196.

RUF, C.; FIEDLER, K. (2000) Trail following as a rare phenomenon among non-social lappet moth larvae (Lepidoptera: Lasiocampidae). *Entomologia generalis* 25, 17-25.

RUF, C.; COSTA, J.T.; FIEDLER, K. (2001) Trail-based communication in social caterpillars of *Eriogaster lanestris* (Lepidoptera: Lasiocampidae). *Journal of Insect Behavior* 14, 231-245.

RUF, C.; KORNMAIER, B.; FIEDLER, K. (2001) Continuous long-term monitoring of daily foraging patterns in three species of lappet moth caterpillars (Lepidoptera: Lasiocampidae). *Nota lepidopterologica* 24(3), 87-99.

RUF, C.; FIEDLER, K. (2002) Plasticity in foraging patterns of colonies of the small eggar moth, *Eriogaster lanestris* (Lepidoptera: Lasiocampidae). *Oecologia* 131, 626-634.

RUF, C.; FIEDLER K. (2002) Tent-based thermoregulation in social caterpillars of *Eriogaster lanestris* (Lep., Lasiocampidae): Behavioral mechanisms and physical features of the tent. *Journal of Thermal Biology* 27, 493-501.

Other publications

RUF, C.; SCHREIER, H-P.; FIEDLER, K. (2002) Zur Entwicklung und Morphologie der Präimaginalstadien und Falter von *Bufoidia pittawayi* Wiltshire & Legrain, 1997 (Lepidoptera, Lasiocampidae). *Entomologische Zeitschrift* 112, 114-119.

Manuscripts submitted to peer reviewed journals

RUF, C.; FIEDLER, K.: Colony survivorship of social caterpillars in the field: A case study of the small eggar moth (Lep., Lasiocampidae).

FIEDLER, K.**; RUF, C.**: *Araschnia levana* larvae (Lep., Nymphalidae) do not accept *Humulus lupulus* (Cannabaceae) as food plant.

RUF, C.; FIEDLER, K.: The mechanisms of trail-based communication in a social caterpillar: Trail marking and recruitment.

RUF, C.; FREESE, A.; FIEDLER, K.: Larval sociality in three species of central-place foraging lappet moths (Lasiocampidae): A comparative survey.

Published conference abstracts

RUF, C.; KORNMAIER, B.; FIEDLER, K. (1999) Aktivitätsmuster und Synchronität bei sozialen Raupen von *Eriogaster lanestris* (Lepidoptera: Lasiocampidae). 29th Annual Meeting of the Gesellschaft für Ökologie, abstract book, 137.

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Appendix

Technical details of the use of light barriers under field conditions (→Chapter 6)

The following section contains additional information to the specifications made in Chapter 6 which give insight into the technical background of the electronic components and the exact realization of the experimental design.

IS471F (SHARP) is a built-in signal processing circuit for a light modulation system. The light detecting element and the signal processing circuitry is integrated on a single chip. Since the spectral sensitivity is maximal at λ=940nm I used LD274 light emitting diodes that show a peak emission at this wavelength.

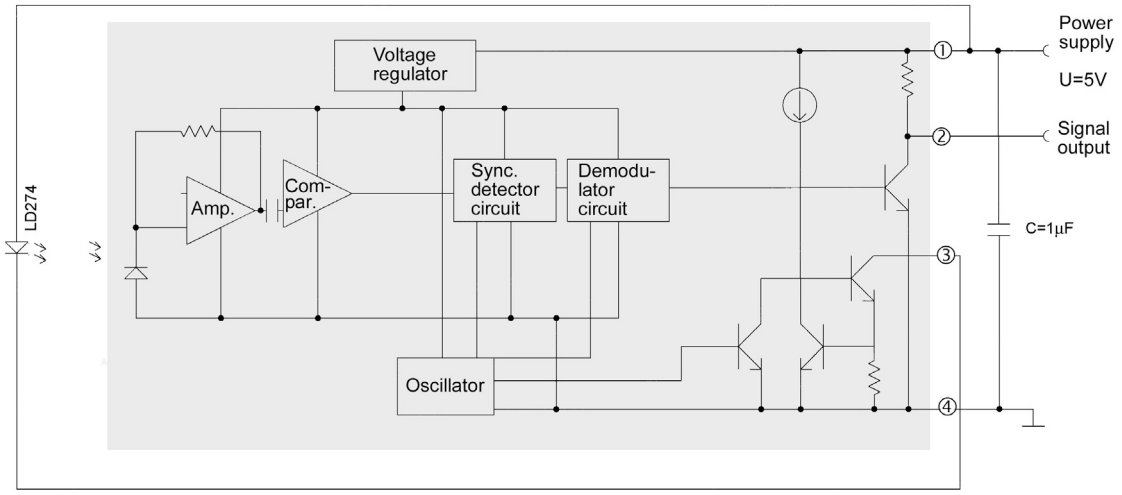


Fig. 1 – Basic circuit of the modulated IR detector IS471F (SHARP). The internal connection diagram is shaded in gray (all information from the data sheet of SHARP).

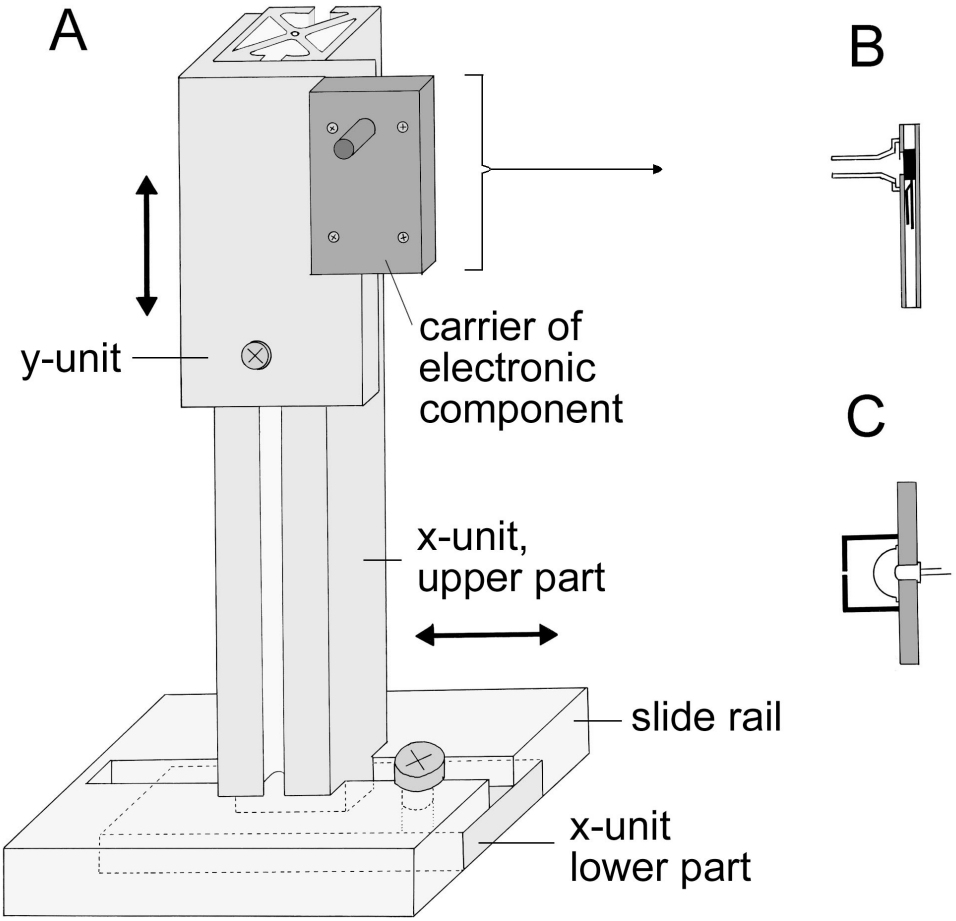
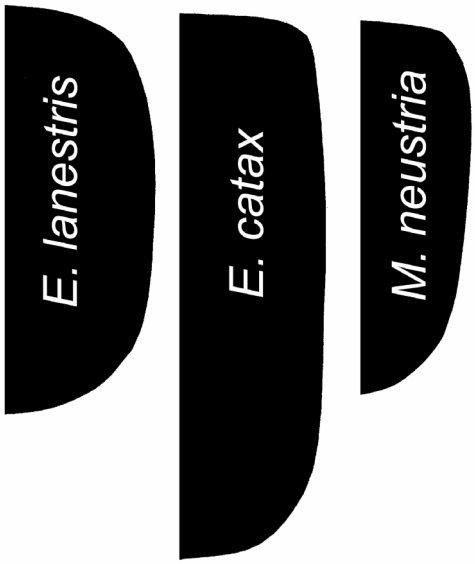


Fig. 2 – Drawing of the xy-unit used for a precise justification of emitters and detectors. Total height of the construction: 25cm. **A** 3D overview. y-unit and both parts of x-unit made of aluminum, slide rail consists of PVC with mill-cut rail. **B** Carrier of light detector. Material: Three PVC layers, inner tile excised in form of the component, front tile with 3mm opening and tube **C** Carrier of light emitting diode with plastic lens and perforated dressing. Material: Solid PVC with 5mm drill hole.

Geometry and volume of eggs of *Eriogaster lanestris*, *E. catax*, and *Malacosoma neustria* (Chapter 11)

Measuring egg size with the help of a stereomicroscope poses an optical problem, especially when measuring the diameter of the eggs. If eggs do not form a regular ellipsoid but are more conically formed, egg volume is overestimated since the viewer measures the maximum diameter by focusing. Fig. 3 shows the half-forms of the eggs of the three species. It is obvious that eggs of *M. neustria* are particularly susceptible to miscalculations of size since they are a bit tapered whereas those of *E. lanestris* are more barrel-like and those of *E. catax* are only tapered at the very end.



Mean diameter:
E. lanestris: 0.75
E. catax: 0.73
M. neustria: 0.64

Mean length:
E. lanestris: 1.17
E. catax: 1.56
M. neustria: 1.07

Fig. 3 – Half-profiles of the eggs of *E. lanestris*, *E. catax*, and *M. neustria* drawn from microphotographs taken at a stereomicroscope equipped with a digital camera. These half-profiles were used for the calculation of egg volume in chapter eleven.

Comparing egg size is normally done in the ecological literature by calculating regular ellipsoids based on the length and the diameter (width) of the eggs.

García-Barros (2000)[1] additionally uses a cubicroot transformation which is said to ameliorate the results if egg shapes are not ideal ellipsoids.

Table 1 shows that this approximation is an oversimplification and results in a strong overestimation of the volume of *M. neustria* eggs.

Table 1. Comparison of egg size calculated with a software FormZ, Ver. 2.9 (AutoDesSys) based on the real shapes of the eggs and the calculation based on the width and length of the eggs according to García-Barros (2000). Formula: egg size = $\sqrt[3]{0.5236 \times d^2 \times h}$ (d = egg diameter, h = egg length). Volume of regular ellipsoid: $V = \frac{4}{3}\pi r_1^2 r_2$ (r_1=radius short axis, r_2=radius long axis). Relative egg size always refers to the volume / size of the largest eggs, i.e. those of *E. catax*.

	E. lanestris	*E. catax*	*M. neustria*
Linear (=cubicroot) size estimation [mm]	0.70	0.76	0.61
Relative egg size	92%	100%	80%
Volume of regular ellipsoid [mm³]	0.34	0.44	0.23
Relative egg size	79%	100%	53%
True egg volume [mm³]	0.52	0.73	0.25
Relative egg size	71%	100%	34%

Probably, conical egg shapes like in *M. neustria* are a rare phenomenon, since they are caused by strong bending of eggs in the whole spiraled egg cluster. This effect is less pronounced in *Eriogaster* where egg clusters are not twisted around the twigs (*E. catax*) or the cluster has the form of an open spiral (*E. lanestris*).

Nevertheless, I assume that such large variations as shown above, which distort relative differences between the species, render results of existing studies comparing egg size across species with very different egg geometries questionable.

[1] García-Barros, E. (2000) Body size, egg size, and their interspecific relationships with ecological and life-history traits in butterflies (Lepidoptera: Papilionidea, Hesperioidea). Biological Journal of the Linnean Society 70, 251-284.

Species list *Eriogaster*

Table 2. List of *Eriogaster* species including Europe, Asia Minor and the territory of the former USSR. Possibly, there are additional species in China.

Species	Reference
Eriogaster acanthophylli (Christoph, 1882)	Zolotuhin 1995
Eriogaster arbusculae Freyer, 1849	Trawöger 1977
Eriogaster catax (Linnaeus, 1758)	ProNatura 2000
Eriogaster czipkai Lajonquiere, 1975	de Freina 1999
Eriogaster daralagesis Zolotuhin, 1991	Dubatolov & Zolotuhin 1992
Eriogaster henkei (Staudinger, 1879)	Zolotuhin 1995
Eriogaster lanestris (Linnaeus, 1758)	Balfour-Browne 1933
Eriogaster neogena (Fischer de Waldheim, 1824)	Zolotuhin 1995
Eriogaster nippei de Freina, 1988	Ortner 1994, de Freina 1999
Eriogaster pfeifferi Daniel, 1932	Daniel 1932
E. p. pfeifferi = *Eriogaster amygdali* Wiltshire, 1941	Talhouk 1975
E. p. talhouki = *Eriogaster amygdali talhouki* Wiltshire, 1975	Talhouk 1975
Eriogaster philippsi Bartel, 1904	Bartel 1904, Talhouk 1940
Eriogaster reshoefti Schulte & Witt, 1975	de Freina 1999
= *E. amygdali reshoefti* Schulte & Witt, 1975	de Freina 1999
Eriogaster rimicola (Denis & Schiffermüller, 1775)	Lunak 1937

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List of gregarious Lasiocampidae (genera)

The following table gives a list of genera within the Lasiocampidae with at least one species reported to have gregarious caterpillars. C = Central, N = North, S = South, E = East. All generic names were checked for validity according to Fletcher & Nye (1982).

Genus	Distribution	Reference
Anadiasa	Africa	Aurivillius 1927
Bombycomorpha	Africa	Aurivillius 1927, Taylor 1949
Catalebeda	Africa	Costa & Pierce 1997
Diapalpus	Africa	Aurivillius 1927
Eriogaster	Europe, Asia	Ebert 1994
Eutachyptera	S + C America	Sallé 1856, Comstock 1957
Gloveria	S + C America	Fitzgerald & Underwood 1998
Gonometa	Africa	Austara et al. 1971
Lebeda	SE Asia	Holloway 1987
Malacosoma	Europe, Asia, N America	Fitzgerald 1995
Mallocampa	Africa	Vuattoux 1991
Metanastria	India, China, SE Asia	Aherkar et al. 1997
Mimopacha	Africa	Aurivillius 1927
Rhinobombyx	Africa	Costa & Pierce 1997
Schausinna	Africa	Costa & Pierce 1997
Taragama	Africa	Aurivillius 1927
Tolype	S America	Arce de Hamity & Neder de Roman 1981
Trabala	SE Asia	Murphy 1990

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Erklärung

Hiermit erkläre ich, daß ich die vorliegende Arbeit selbständig verfaßt und dabei keine anderen als die angegebenen Hilfsmittel verwendet habe.

Ferner erkläre ich, daß ich diese Arbeit weder einer anderen Prüfungsbehörde vorgelegt noch anderweitig mit oder ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

Bayreuth, den 1. Dezember 2002

Claudia Ruf

»Larvæ cohabitant in Societate sub tentoriis cellulosis, unde migrant pabulaturæ, redeuntque per foramina ramis parallela.«
(C. v. Linné: Systema naturae, XIIth ed., 1767)

»Obwohl auf dem Gebiete der Tiersoziologie in den letzten zwei Dezennien eine reiche und fruchtbare Literatur über Zustandekommen und Einteilung soziologischer Erscheinungen im Zusammenhange mit Untersuchungen, welche die bisherige Vorstellung vom Wesen des Instinktes wesentlich verändert haben, entstanden ist, liegen fast keine Beobachtungen über den biologischen Wert soziologischer Tatsachen vor.«
(H. Rebel: Über den biologischen Wert von Larvensozietäten, 1931)

»Largely unrecognized, the gregarious behavior of caterpillars is changing the way entomologists think about social insects. (…) More than mere aggregates of individuals, these species appear to have exploited the possibilities inherent in grouping – potentials for building shelters, behaviorally thermoregulating, warding off enemies and finding food. Social caterpillars are in many ways very different social organisms than their better known relatives, but this difference underlies an important lesson about what means to be social, ironically pointing the way to commonalities across the sociality spectrum.«
(J.T. Costa: Caterpillars as social insects